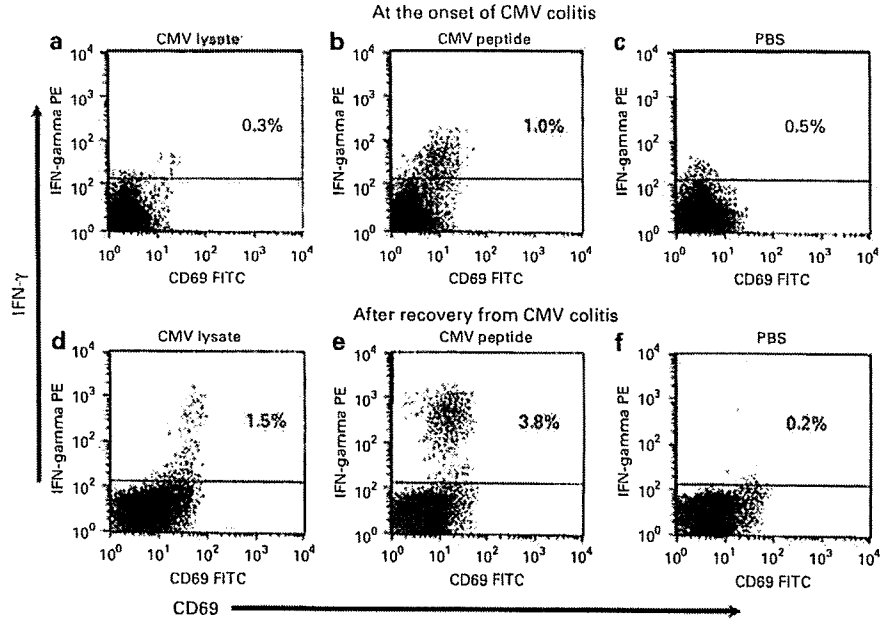

Figure 4 Intracellular cytokine assay in a patient with CMV colitis (UPN2). The samples were taken at the onset of CMV colitis (a–c) and after recovery from CMV colitis (d–f). The numbers of IFN-γ-producing cells on lysate stimulation (a, d) and peptide stimulation (b, e) both increased after recovery from CMV colitis. (c) and (f) are negative controls.

onset and were undetectable for the other two patients, which remained negative until day 90 for UPN1. The mean number of IFN-γ+ cells subsequently increased to 19 (5–38)/μl after recovery from CMV disease (Figures 2, 4a and d). Among the patients who did not require antiviral therapy, the IFN-γ-producing cells were all >10/μl at day 60.

When stimulated with CMV peptide, IFN-γ-producing cells numbered 8 (0–16)/μl at the time of disease onset with a subsequent increase to 47 (15–95)/μl after recovery from CMV disease (Figures 4b and e).

Regarding the phenotype of IFN-γ-producing cells, median of 81% (76–100) were CD4+ and <20% were CD8+ upon stimulation by CMV lysate. The staining of IFN-γ was brighter in CD4+ than in CD8+ cells and CD69 was positive for both CD4+ and CD8+ fraction. IFN-γ-producing cells were CD69 low positive and median of 42% (25–68) were CD8+, while the rest were CD8−/CD4− phenotype upon CMV peptide stimulation.

Discussion

Our results showed that it is difficult to predict CMV infection by the number of CMV-specific CTL alone as this did not correlate with the incidence and severity of CMV infection. While UPN1 and UPN2 developed CMV colitis after the recovery of sufficient number of CTL, UPN6, UPN7 and UPN8 did not require antiviral therapy despite low CMV-specific CTL. These results showed that CMV disease could occur after HSCT even in patients with >10/μl CMV-specific CTL as evaluated by tetramer assay, which has been considered to be sufficient to protect against CMV infection.[5,7]

CMV-specific CTL emerged immediately following the detection of antigenemia in most patients, suggesting that CMV infection can be a trigger for the recovery of CMV-specific immunity. However, UPN9 had recovery of CMV-specific CTL at day 60 even though his CMV antigenemia and CMV DNA as evaluated by PCR were negative throughout the course.

On the other hand, intracellular analysis revealed that IFN-γ production in both CD4+ and CD8+ T lymphocytes was depressed in patients with high antigenemia or CMV disease and this had subsequently recovered at disease resolution. Functional analysis methods for CMV-specific immune response by flow cytometry have been established,[16] and it was reported that patients who developed CMV disease after SCT had no detectable IFN-γ production by CD3+/4+ T-cells upon CMV AD-169 antigen stimulation.[17] It has also been demonstrated that levels of IFN-γ-producing CD4+ cells less than one cell/μl and CD8+ less than three cells/μl upon stimulation by CMV-infected autologous dendritic cells are not protective against recurrent infection.[18] As assessed by IFN-γ ELISPOT assay, the threshold level for protection against CMV reactivation was estimated as over one cell/μl peripheral blood upon CMV pp65 peptide stimulation.[19] The number of IFN-γ-producing cells upon CMV lysate stimulation were above ten cells/μl among patients whose antigenemia was <10/50 000 cells in our study, which may be sufficient for protection against CMV reactivation. It is difficult to determine the exact threshold level for protection against CMV since IFN-γ production differs among various stimulating agents. Also the magnitude of response is higher in the cytokine flow cytometry assay while the cytokine flow cytometry assay was less likely than the ELISPOT assay to detect low-level responses.[20]

Several studies on HIV-infected patients have shown the availability of analyzing the phenotype and other cytokine production of virus-specific T-cells such as IL-2, TNF-α.[21–23] It has been demonstrated that virus-specific T-cells, which produce both IFN-γ and IL-2 are important in virus-specific immunity, and that IFN-γ/IL-2 secreting CD8+ T-cells were CD45RA−/CCR7− phenotype and correlated with that of proliferating T-cells, whereas single IFN-γ-secreting cells were either CD45RA−/CCR7− or CD45RA+/CCR7−.[22] Another study has shown that immunorestored patients had increased levels of circulating CMV-specific CD8+ T-cells with 'early' (CD27+/CD28+/CD45RA+, CD27+/CD28+/CD45RA−) and 'intermediate' (CD27−/CD28+/CD45RA−) phenotype.[23] Only IFN-γ production was assessed in our study, however higher-order flow cytometry might have added more discriminatory value. Foster *et al*.[24] demonstrated that CMV-specific CD4+ T-helper cells show the same reconstitution kinetics as CD8+ CTL. Thus, functional analysis of lymphocytes upon lysate stimulation that can be used to assess both CD4+ and CD8+ cells is a useful tool for monitoring T cell immunity against CMV in patients after HSCT. This method is more widely applicable than peptide stimulation or tetramer assay, since it is not restricted to HLA or a single epitope. However, peptide stimulation and tetramer assay may still be a major procedure in the analysis of CD8+ T-cells, since tetramers are widely applied to adoptive immunotherapy of CMV[25] and the dominant population of IFN-γ-producing cells upon lysate stimulation was CD4+. Previous study has demonstrated that flow cytometry following stimulation of PBMC with pp65 and immediate early (IE)-1 peptide pools consisted of 15-aa peptides was highly sensitive and specific in predicting the presence of recognized epitope in the respective proteins.[26] Furthermore, it has been shown that IE-1-specific responses were more important in protective immunity than pp65-specific responses in heart and lung transplant recipients.[27] The stimulation with comprehensive peptide pools might have better assessed both functional CD4+ and CD8+ T-cell responses. Further study is needed to identify whether IE-1 is more important than pp65 in allogeneic HSCT patients, and the significance of IE-1 in Japanese population with low allele frequency of HLA-A1 (1.8%), -B7 (5.2%) or -B8 (<1%),[15] which is known to present IE-1 epitopes.

It is likely that the patients who did not have CMV reactivation despite low CMV-specific CTL had sufficient T-cell immune-recovery against CMV since the number of intracellular IFN-γ positive cells upon CMV lysate stimulation was as high as that in patients who had recovered from CMV reactivation. As for CD8+ T cells in these patients, CTL against other CMV-epitopes besides NLV might have helped to protect against CMV. It is reported that the recovery of CMV specific T-cells is earlier in patients who received reduced-intensity conditioning compared to conventional regimen and this was delayed by the use of ATG.[19,28] Additionally, the graft source and CD3+ T-cell dose significantly influence the recovery of CMV-specific immunity.[28] The difference of immune recovery according to the conditioning regimen and graft source was not demonstrated in this study, probably due to heterogeneous patients and small sample size. Functional depression of the lymphocytes due to corticosteroid for GVHD seems to be the major cause of CMV infection as documented in all patients with high antigenemia. Moreover, 75% of the patients with CMV disease were receiving more than 1 mg/kg/day of methylprednisolone (mPSL), while among those who did not require antiviral therapy, only 13% had received 1 mg/kg/day or more mPSL. The influence of corticosteroid on the number of CMV-specific CTL is controversial. Some studies have reported that a significant reduction of CMV-specific CTL occurred with corticosteroid therapy.[6,8] Others have shown that the frequency and the absolute number of CMV-specific CD8+ T cells were similar in patients receiving corticosteroids and those who didn't, while the CMV-specific CD8+ T cells showed decreased cytokine production.[10,11] Our result was consistent with the latter observation that while the number of CMV-specific CTL does not decrease significantly with corticosteroid therapy, IFN-γ production of CMV-specific CTL is severely suppressed. Therefore, concomitant assessment of T-cell function is essential in patients after HSCT, especially in those who are receiving corticosteroid therapy.

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Infectious complications in chronic graft-versus-host disease: a retrospective study of 145 recipients of allogeneic hematopoietic stem cell transplantation with reduced- and conventional-intensity conditioning regimens

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Abstract: To assess infectious complications associated with chronic graft-versus-host disease (cGVHD) after allogeneic hematopoietic stem cell transplantation (HSCT) with reduced- and conventional-intensity conditioning regimens (RIC, $n = 91$; CIC, $n = 54$, respectively), we retrospectively analyzed data from 145 consecutive patients with cGVHD after allogeneic HSCT from a human leukocyte antigen-matched related or unrelated donor. In the present retrospective analysis, 57% (83/145) of patients with cGVHD developed infections, with a mortality rate of 27% (22/83). The incidences of bacteremia ($n = 28$), central venous catheter-related infections ($n = 11$), bacterial pneumonia ($n = 4$), invasive aspergillosis ($n = 7$), and adenoviral hemorrhagic cystitis ($n = 8$) were significantly higher in patients with prednisolone dose ≥ 1 mg/kg at the time of diagnosis of cGVHD. The present results suggest that infections associated with cGVHD, especially after high-dose prednisolone, are predictive of poor outcome regardless of whether the patient received RIC or CIC.

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Key words: infectious complication; chronic graft-versus-host disease; allogeneic hematopoietic stem cell transplantation; reduced-intensity conditioning; HLA-matched donor

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Infectious complications contribute to morbidity and mortality following allogeneic hematopoietic stem cell transplantation (HSCT). Well-known factors affecting susceptibility to infections include donor type, conditioning regimen, development of graft-versus-host disease (GVHD), and environmental factors. Reduced-intensity conditioning (RIC) regimens are thought to lower the risk of infections because they involve relatively little damage to vital organs (1). However, our experience indicates that with both RIC and conventional-intensity conditioning (CIC) regimens, the incidence of bacterial infections during neutropenia and *Aspergillus* infections is high after allogeneic HSCT (2, 3). Thus, it appears that RIC alone is not sufficient to improve the safety of allogeneic HSCT.

GVHD and the treatment of GVHD with immunosuppressive drugs are also well-known predominant risk factors for the development of opportunistic infections (4–6). In the case of acute GVHD, inpatients can be given comprehensive prophylaxis, including environmental control, to prevent infections over the short term. In contrast, chronic GVHD (cGVHD) is most often a late complication of allogeneic HSCT, and is usually treated on an outpatient basis. Consequently, the resources that can be used to control infections in patients with cGVHD are limited, and prophylaxis should be considered as a long-term approach, taking into account the safety and emergence of drug-resistant pathogens. In Japanese patients, the incidence of cGVHD after allogeneic HSCT is reportedly as high as 50%, with 20% of those who develop cGVHD contracting concurrent infections (7). At present, more transplantation procedures are being performed with peripheral blood stem cell (PBSC) products, in older patients, and with

unrelated donors. The available evidence suggests that all of these factors would result in greater numbers of patients with cGVHD. Thus, management of cGVHD is one of the greatest challenges to physicians practicing HSCT.

In the present study, we evaluated infectious complications associated with cGVHD in patients who received an RIC or a CIC regimen before undergoing PBSC transplantation (PBSCT) from a human leukocyte antigen (HLA)-matched relative (related PBSCT) or bone marrow transplantation (BMT) from an HLA-matched unrelated volunteer (unrelated BMT).

Patients and methods

Patient characteristics

We retrospectively analyzed data from 145 consecutive adult patients with hematologic malignancies who had received allogeneic HSCT with an RIC ($n = 91$) or CIC ($n = 54$) regimen between January 2000 and December 2004 at our institution. All of these 145 patients had sustained engraftment, had survived for >100 days following transplantation, and had developed cGVHD. The following types of patients were excluded: patients who suffered from disease progression before the development of cGVHD and received donor lymphocyte infusion, and patients with a history of previous allogeneic HSCT. Significant differences were observed between the RIC and CIC groups in terms of the age of the patients and donors, the gender of the patients, diagnosis, disease risk (8), time from diagnosis to transplantation, donor type and source of stem cells, and GVHD prophylaxis. The patient characteristics are summarized in Table 1. Typing for HLA-A, -B, and -DR antigens of the donor and recipient was performed using low-resolution DNA typing. The frequency with which allogeneic PBSCT is performed in Japan has been increasing since it became eligible for reimbursement from health insurance organizations in the year 2000, and our banking system only approves donation of bone marrow. The clinical characteristics of cGVHD, including use of immunosuppressive drugs at diagnosis and initial treatment, are summarized in Table 2. The present study was approved by the Ethics Committee of our institution, and all 145 subjects provided informed consent.

Conditioning regimen and supportive care

The CIC regimen consisted of cyclophosphamide (CY, 120 mg/kg), in combination with either 12 Gy total-body irradiation (TBI, $n = 25$) or busulfan (BU, 16 mg/kg; $n = 29$). The RIC regimen consisted of BU (8 mg/kg) in combination with either fludarabine (Flu, 180 mg/m^2; $n = 70$) or 2-chlorodeoxyadenosine (2-CdA, 0.66 mg/kg; $n = 21$); 14 patients received either anti-thymocyte globulin (ATG, 5–10 mg/kg; $n = 6$) or 4 Gy TBI ($n = 8$). All patients received cyclosporine (CSP, 3 mg/kg/day; $n = 137$) or tacrolimus (TAC, 0.03 mg/kg/day; $n = 8$), with ($n = 78$) or without ($n = 67$) short courses of methotrexate (MTX; related PBSCT, 10 mg/m^2 on day 1, and 7 mg/m^2 on days 3 and 6; unrelated BMT, 10 mg/m^2 on days 3, 6, and 11) as GVHD prophylaxis. All patients received prophylactic ciprofloxacin (200 mg orally 3 times daily) for prevention of infections until neutrophil recovery. Trimethoprim-sulfamethoxazole (80 mg of trimethoprim once daily) was administered for the prevention of *Pneumocystis* pneumonia and encapsulated bacterial infection, from the first day of the conditioning regimen until day 3, and from day +30 until 6 months after transplantation, or for prolonged periods in patients with cGVHD. Patients also received oral or intravenous fluconazole (100 mg once daily) for prevention of infection by *Candida* species, and low-dose acyclovir (600 mg until engraftment, and then 100 mg/day orally), starting at the same time as the conditioning regimens and continuing until cessation of administration of immunosuppressive drugs (9). Cytomegalovirus (CMV) antigenemia was monitored weekly until cessation of the administration of immunosuppressive drugs. Testing for CMV antigenemia consisted of direct immunoperoxidase staining of leukocytes with a peroxidase-labeled monoclonal antibody. Quantitative real-time polymerase chain reaction was not performed.

Definition of outcome

Patients with grades II–IV acute GVHD were treated with prednisolone (PSL) according to a standard regimen (10). Chronic GVHD was assessed and graded according to the standard criteria (11). The diagnosis and staging of cGVHD were also assessed according to the working report published by the National Institutes of Health Consensus Development Project (12). Relapse was defined either by morphologic evidence of the disease in the peripheral blood, marrow, or extramedullary sites, or by recurrence and persistence of pre-transplant chromosomal abnormalities in cytogenetic analysis of the marrow cells.

Infectious complications

A documented infection was defined as signs and symptoms associated with microbiological documentation of a pathogen from the site of infection. Culture-documented bacteremia, fungemia, or viremia was considered to be a definite infection, regardless of symptoms. On the other hand, clinical infection was defined as signs or symptoms consistent with an infection, but without microbiological confirmation. Central venous catheter (CVC)-related

Patient characteristics and transplant outcomes

	RIC ($n = 91$)	CIC ($n = 54$)	P
Median age of patients (range)	55 (26–68)	37 (18–53)	< 0.0001
Median age of donors (range)	50 (17–69)	34 (19–54)	< 0.0001
Male/female patient	57[1]/34	22/32	0.015
Female donor for male patient	19	10	0.83
Diagnosis AML (+ MDS)	27 (9)	17 (4)	0.0029
MDS	17	4	
CML	7	12	
ALL	1	8	
ML	36	13	
Others[2]	3	0	
Disease risk group (standard/advanced)[3]	14/77	21/33	0.0023
Median time interval[4] (range), (months)	19 (2–178)	10 (1–100)	0.014
KPS[5] $\leq$ 80%	10	5	0.41
HCT-SCI[6] $\geq$ 2	13	7	0.99
Prior infectious complications	6	3	0.99
Prior autologous transplantation	5	2	0.99
Donor type and source of stem cells			
Related PBSC/Unrelated BM	82/9	34/20	0.0002
GVHD prophylaxis			
CSP orTAC alone/MTX with CSP orTAC	66/25	1/53	< 0.0001
Acute GVHD grade II/III/IV	24/23/3	18/8/2	0.072
Median onset day (range) of grades II–IV acute GVHD[7]	39 (12–97)	32 (14–91)	0.48
Prior use of PSL for acute GVHD			
0.5–<1.0/1.0–<2.0/ $\geq$ 2.0 mg of PSL/kg	5/34/18	4/13/9	0.27
Relapse/progressive disease following cGVHD	16	10	0.99
Cause of death	30	20	0.27
Infection	15[8]	7[8]	
Chronic GVHD	9[8]	8[8]	
Lungs/gastrointestinal tract/MOF/Others[9]	3/1/3/2	3/3/2/0	
Others[10]	3	6	
Progression	8	2	
Median follow-up (range), (months)	39 (5–73)	45 (15–79)	0.20

[1]Number of patients, unless indicated otherwise.
[2]Others = myelofibrosis ($n = 1$), chronic lymphocytic leukemia ($n = 1$), and multiple myeloma ($n = 1$).
[3]Patients who were considered standard risk with a diagnosis of AML + MDS or AML, or ALL in first complete remission, CML in first chronic phase, or untreated refractory anemia in MDS. All other conditions were considered to indicate advanced risk.
[4]Time from diagnosis to transplantation.
[5]KPS was evaluated before the start of the conditioning regimen, and was graded according to Karnofsky performance status score.
[6]HCT-SCI was evaluated before the start of the conditioning regimen, and was graded according to hematopoietic cell transplantation-specific comorbidity index (ref. 8).
[7]Time from occurrence of grades II–IV acute GVHD to transplantation.
[8]Total number of patients differs because 8 patients (RIC, 5; CIC, 3) died of both infection and chronic GVHD.
[9]Others = renal ($n = 1$) and liver ($n = 1$).
[10]Others = RIC: cerebral infarction ($n = 1$), secondary hepatocellular carcinoma ($n = 1$), infection following secondary allogeneic cord blood stem cell transplantation; CIC: acute myocardial infarction ($n = 1$), cerebral infarction ($n = 1$), drug-induced interstitial pneumonia ($n = 1$), infection following chemotherapy ($n = 1$), and suicide ($n = 2$).
RIC, reduced-intensity regimen; CIC, conventional-intensity regimen; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; CML, chronic myeloid leukemia; ALL, acute lymphoblastic leukemia; ML, malignant lymphoma; PBSC, peripheral blood stem cell; BM, bone marrow; CSP, cyclosporine; TAC, tacrolimus; MTX, methotrexate; GVHD, graft-versus-host disease; PSL, prednisolone; cGVHD, chronic graft-versus-host disease; MOF, multiple organ failure.

Table 1

Clinical characteristics of cGVHD

	RIC	CIC	*P*
Median onset day (range)[1]	100 (79–479)	109 (93–348)	0.51
Limited/extensive	5/86	1/53	0.41
De novo/quiescent/progressive	27/38/26	24/9/21	0.16
KPS score 1/2/3	61/16/8	46/2/2	0.045
Skin score 1/2/3	27/33/8	17/12/3	0.15
Mouth score 1/2/3	40/29/3	23/9/1	0.87
Eyes score 1/2/3	30/14/7	15/9/1	0.38
Gastrointestinal tract score 1/2/3	28/4/12	19/1/7	0.84
Liver score 1/2/3	7/23/44	6/13/24	0.89
Lungs score 1/2/3	6/8/4	8/7/1	0.26
Joints and fascia score 1/2/3	13/2/0	8/5/1	0.13
Genital tract score 1/2/3	1/0/0	0/0/0	0.99
Eosinophilia $>0.5 \times 10^9$/L	30	22	0.37
Platelets $<100 \times 10^9$/L	26	20	0.36
Others[2]	5	2	0.39
Immunosuppressive drugs at diagnosis of cGVHD			
CSA/TAC	66/3	37/3	0.76
<0.5/0.5–<1.0/1.0–<2.0/ ≥ 2.0 mg of PSL/kg	17/9/11/2	9/2/3/1	0.57
Initial treatment for cGVHD			
Addition or increased dose of CSA/TAC	69/7	41/4	0.99
<0.5/0.5–<1.0/1.0–<2.0/ ≥ 2.0 mg of PSL/kg	18/14/15/4	10/7/6/2	0.74
Median follow-up from diagnosis of cGVHD (range) (months)	39 (5–73)	45 (15–79)	0.26

[1]Time from occurrence of cGVHD to transplantation.
[2]Others = pleural effusion ($n = 4$), pericardial effusion ($n = 3$), ascites ($n = 3$), and polymyositis ($n = 1$).
RIC, reduced-intensity regimen; CIC, conventional-intensity regimen; cGVHD, chronic graft-versus-host disease; KPS, Karnofsky performance status; CSP, cyclosporine; TAC, tacrolimus; PSL, prednisolone.

Table 2

infections consisted of exit site infections without bacteremia. Bacterial pneumonia was included in the category of definite infections, and was diagnosed by chest x-ray examination or computed tomography (CT) and identification of a bacterial pathogen on culture of sputum, bronchoalveolar lavage fluid, pleural fluid, or blood specimen. Fungal infections, including proven or probable invasive fungal infections, were diagnosed by identification of a fungal pathogen on culture or *Aspergillus* antigen and CT examination according to consensus criteria (13). Pneumonia of unknown origin was included in the category of undefined pneumoniae, which were diagnosed by chest x-ray and/or CT. There was no significant difference in CMV serostatus between the RIC and CIC groups (data not shown). A polymicrobial infection of 1 organ or several adjacent organs was considered to be a single infection. Death associated with a documented infection was defined as the death of a patient with findings consistent with an infection, or as detection of the pathogen in an autopsy specimen.

Statistical analysis

Comparisons of variables were performed using the 2-tailed Fisher exact test or the χ^2 test. Continuous variables were compared by the Mann–Whitney *U*-test. All *P*-values were 2-sided, and the type I error rate was fixed at $P < 0.05$.

Results

Transplant outcomes

The transplant outcomes are summarized in Table 1. Twenty-two patients (RIC, $n = 15$; CIC, $n = 7$) died of infections, of whom 8 patients (RIC, $n = 5$; CIC, $n = 3$) died of

both infections and chronic GVHD, with cGVHD at a median follow up of 40 months from transplantation (RIC, 39 vs. CIC, 45 months). The median onset of cGVHD was 112 days (RIC, 100 vs. CIC, 109 days), and 47 patients (RIC, $n = 26$; CIC, $n = 21$) developed progressive-type cGVHD at a median follow up of 32 months from diagnosis of cGVHD (RIC, 39 vs. CIC, 45 months). The severity of the Karnofsky performance status (KPS) score was significantly greater in the RIC group ($P = 0.045$).

Infectious complications

A total of 134 infectious episodes occurred in 83 patients (RIC, 51 vs. CIC, 32; $P = 0.73$), as shown in Table 3. Of these, 28 patients (RIC, 18 vs. CIC 10; $P = 0.83$) developed bacteremia, the causative organisms (43 positive cultures) of which are summarized in Table 4. Gram-positive bacteremia (27 positive cultures) was more common than gram-negative bacteremia (16 positive cultures). The bacteremia was caused by 2, 3, and 4 types of organisms in 4, 4, and 1 patient, respectively. The incidence of bacteremia was significantly higher in patients with the following factors: cGVHD including progressive types ($n = 15$, $P = 0.0027$), a KPS score ≥ 2 ($n = 11$, $P = 0.0062$) and a gastrointestinal (GI) score ≥ 2 ($n = 13$, $P < 0.0001$); PSL dose ≥ 1 mg/kg at the time of diagnosis ($n = 9$, $P = 0.00090$) and for the initial treatment of cGVHD ($n = 11$, $P = 0.0050$). CVC-related infections ($n = 11$) were caused by *Staphylococcus epidermidis* ($n = 4$), *Staphylococcus* species ($n = 2$), *Stenotrophomonas maltophilia* ($n = 2$), *Acinetobacter iwoffii* ($n = 1$), *Corynebacterium* species ($n = 1$), or methicillin-resistant *Staphylococcus aureus* (MRSA, $n = 1$). The incidence of CVC-related infections was significantly higher in patients with PSL dose ≥ 1 mg/kg at the time of diagnosis of cGVHD ($n = 4$, $P = 0.026$). Bacterial pneumonia was observed in 4 patients, and the isolated organisms were as follows: *Pseudomonas aeruginosa* ($n = 1$), *Hemophilus influenzae* ($n = 1$), *S. epidermidis* ($n = 1$), and *Staphylococcus* species ($n = 1$). The incidence of bacterial pneumonia ($n = 4$) was significantly higher in patients with PSL dose ≥ 1 mg/kg at the time of diagnosis ($n = 3$, $P = 0.0051$) and for the initial treatment of cGVHD ($n = 3$, $P = 0.021$). Invasive aspergillosis (IA) and *Candida* infections developed in 7 and 3 patients, respectively. All patients with IA had been given ≥ 0.5 mg of PSL/kg at the time of diagnosis of cGVHD. The incidence

Infectious complications associated with cGVHD

	Total (median onset, range, days)	RIC	CIC	*P*
Bacterial infections				
Bacteremia	28 (175, 104–1629)	18 (5)[1]	10 (2)	0.83
CVC-related	11 (123, 101–1774)	5 (0)	6 (0)	0.33
Pneumonia	4 (311, 101–1045)	3 (2)	1 (1)	0.99
Others[2]	16 (302, 102–1065)	7 (4)	9 (2)	0.11
Fungal infections				
Candida infection	3 (128, 101–358)	1 (0)	2 (0)	0.56
Invasive aspergillosis	7 (181, 112–1232)	6 (0)	1 (0)	0.26
Viral infections				
Adenoviral hemorrhagic cystitis	8 (192, 111–538)	5 (0)	3 (0)	0.99
CMV colitis	1 (343)	0 (0)	1 (0)	0.37
Cutaneous VZV	18 (502, 106–1684)	12 (0)	6 (0)	0.80
Influenza	4 (483, 355–898)	1 (0)	3 (0)	0.15
Others[3]	2 (133, 103–164)	1 (0)	1 (0)	0.99
CMV antigenemia	15 (140, 104–448)	11 (0)	4 (0)	0.42
Pneumoniae of unknown origin	32 (283, 101–1735)	18 (4)	14 (4)	0.41

[1]Number of infectious episodes (number of deaths) is shown.
[2]Others = sepsis of unknown origin (4 episodes), dermatitis (3), hemorrhagic cystitis (2), otitis media (2), meningitis (2), cholecystitis (1), pseudomembranous enterocolitis (1), and urinary tract infection (1).
[3]Others = herpes simplex viral esophagitis (1 episode) and meningitis (1).
cGVHD, chronic graft-versus-host disease; RIC, reduced-intensity regimen; CIC, conventional-intensity regimen; CVC, central venous catheter; CMV, cytomegalovirus; VZV, varicella zoster virus.

Table 3

Bacteremia associated with cGVHD

	RIC ($n = 18$)	CIC ($n = 10$)
Gram-positive organisms	16[1]	11
Staphylococcus epidermidis	7	2
Streptococcus species	2	3
Enterococcus species	3	0
Staphylococcus species	0	3
Bacillus species	0	1
Corynebacterium species	1	0
MRSA	0	1
Gram-positive cocci	3	1
Gram-negative organisms	10	6
Bacteroides species	3	2
Pseudomonas aeruginosa	2	2
Klebsiella species	2	0
Enterobacter species	0	1
Escherichia coli	0	1
Gram-negative rods	3	0

[1]Number of positive cultures.
cGVHD, chronic graft-versus-host disease; RIC, reduced-intensity regimen; CIC, conventional-intensity regimen; MRSA, methicillin-resistant *Staphylococcus aureus*.

Table 4

of IA was significantly higher in patients with cGVHD including a GI score ≥ 2 ($n = 4$, $P = 0.015$), PSL dose ≥ 1 mg/kg at the time of diagnosis ($n = 4$, $P = 0.0037$), and for the initial treatment of cGVHD ($n = 7$, $P < 0.0001$). Eighteen patients developed cutaneous varicella zoster virus (VZV); all responded promptly to acyclovir. Eight patients developed adenoviral hemorrhagic cystitis (HC); 2 of these 8 patients developed continuously complicated lethal bacteremia. The incidence of adenoviral HC was significantly higher in patients with cGVHD including a KPS score ≥ 2 ($n = 5$, $P = 0.0071$) and a GI score ≥ 2 ($n = 4$, $P = 0.026$); PSL dose ≥ 1 mg/kg at the time of diagnosis ($n = 4$, $P = 0.0069$); and for the initial treatment of cGVHD ($n = 5$, $P = 0.0060$). *De novo* CMV antigenemia before or after development of cGVHD was observed in 62 and 15 patients, respectively. Sixteen and 8 patients, respectively, died of bacterial infections and pneumoniae of unknown origin.

Discussion

In the present retrospective analysis, 57% (83/145) of patients with cGVHD developed infections, with a mortality rate of 27% (22/83). Although the limitations of this study were the retrospective study design and the differences in baseline characteristics in both the RIC and CIC groups, these results illustrate the importance of establishing more effective management of infectious complications associated with cGVHD, which are predictive of poor outcome for both RIC and CIC regimens.

In patients with cGVHD, the major source of bacteremia was heterogeneous, gram-positive organisms such as *S. epidermidis* and *Streptococcus* species, which were more common than gram-negative organisms, and bacteremia caused by *Pseudomonas aeruginosa*, including multidrug-resistant *P. aeruginosa*, occurred only in patients with cGVHD involving a GI tract score ≥ 2. Additionally, *Streptococcus pneumonia* sepsis was a risk factor for non-relapse mortality, as reported previously (4), and pneumococcal vaccination of transplant recipients was found to be relatively ineffective in the presence of cGVHD. In other studies with RIC regimens, the incidence of bacteremia appeared to be significantly lower than in the present study, but this may a result of the shorter follow-up periods in those studies (14, 15). Moreover, 29% (7/24) of the present patients with cGVHD involving a GI tract score ≥ 2 had received ≥ 2 mg of PSL/kg before developing cGVHD, and all 7 of these patients developed bacteremia. Although 50% (14/28) of patients with bacteremia received antibiotic

drugs and all 14 of these patients received intravenous immunoglobulin to maintain IgG levels at >400 mg/dL for prophylaxis of encapsulated bacteria and *Pneumocystis*, these results suggest that patients with cGVHD having a GI tract score ≥2, especially after high-dose PSL, are more likely to develop bacteremia than patients with cGVHD not having a GI tract score ≥2. This was probably due to colonization of the GI tract resulting from translocation into the bloodstream or disruption of the ecologic GI equilibrium involving GI bacterial overgrowth (e.g., use of antibiotic decontamination), increased permeability of the GI mucosal barrier (e.g., GVHD-induced mucosal damage), or deficiencies in the host immune defenses (e.g., use of immunosuppressive drugs). Thus, a review of strategies for prevention of bacteremia may lead to improvement of patient outcomes after allogeneic HSCT. That is, in patients with cGVHD having a GI tract score ≥2, restrictions on the use of broad-spectrum antibiotics may help reduce GI bacterial overgrowth, including overgrowth by antibiotic-resistant organisms, resulting from failure of the GI barrier. In contrast, we recognize the difficulty in identifying bacteremia using culturing blood. Our patients were immunocompromised hosts who presented with undifferentiated fever; therefore, blood culture results were often delayed well into the course of empirical therapy. There is a need to develop suitable strategies for screening of bacteremia associated with cGVHD in patients who receive allogeneic HSCT with either RIC or CIC regimens.

Most of the present patients with cGVHD who developed *Candida* infection or IA received ≥0.5 mg of PSL/kg before developing cGVHD and the incidence of IA was significantly higher in patients with cGVHD having a GI score ≥2, especially after high-dose PSL. The number of patients with fungal infections was small, but high-dose PSL may be effective for improving the prophylaxis for such infections. Furthermore, the duration of prophylaxis still remains unclear as randomized clinical trials have yet to be conducted.

All the present patients with adenoviral HC developed grades II–IV acute GVHD and received PSL for GVHD therapy, which differs considerably from what has been reported previously(16). The incidence of adenoviral HC was significantly higher in patients with cGVHD having a KPS score ≥2, a GI score ≥2, and high-dose PSL at the time of diagnosis and for the initial treatment of cGVHD. Although the present study was limited in its ability to detect risk factors for adenoviral HC, because of low patient numbers and lack of prospective investigation of viral infection, the present results suggest that patients who receive high-dose PSL before and after developing cGVHD should be frequently checked for abdominal and urinary symptoms, and that urinary tests should be regularly performed during ongoing use of immunosuppressive drugs. In addition, we identified only 1 patient with cGVHD who suffered from CMV colitis, indicating that it is useful to monitor and treat CMV antigenemia intensively in patients receiving immunosuppressive drugs, especially before development of cGVHD. In contrast, 12% of the present patients with cGVHD developed cutaneous VZV with a median onset of 502 days (range, 106–1684), despite low-dose acyclovir prophylaxis during at least the first year after allogeneic HSCT. Nonetheless, there were no cases of breakthrough VZV infection. This suggests that low-dose acyclovir prophylaxis effectively prevented breakthrough VZV infection, but that reestablishment of antiviral therapy was needed to protect against cutaneous VZV in patients with cGVHD.

In summary, the present data indicate that infections associated with cGVHD, especially after high-dose PSL, are predictive of poor outcome, whether RIC or CIC is used. Accordingly, there is a need for clinical trials to develop new strategies for screening and prevention of infections associated with cGVHD in patients who receive allogeneic HSCT with either RIC or CIC regimens.

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Hyperglycemia During the Neutropenic Period Is Associated With a Poor Outcome in Patients Undergoing Myeloablative Allogeneic Hematopoietic Stem Cell Transplantation

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Background. Recipients of allogeneic hematopoietic stem cell transplantation (HSCT) frequently require support with parenteral nutrition and immunosuppressive drugs, which introduce the risk of hyperglycemia. Van den Berghe et al. showed that the strict glucose control improved the outcome of patients treated in the intensive care unit, and this point was evaluated in this study in a HSCT setting.

Methods. A cohort of 112 consecutive adult patients treated by myeloablative allogeneic HSCT between January 2002 and June 2006 was reviewed retrospectively. Twenty-one patients were excluded due to graft failure, preexisting infectious diseases, preexisting neutropenia or previous allogeneic HSCT. The remaining 91 patients were categorized according to mean fasting blood glucose (BG) level in the neutropenic period after conditioning: normoglycemia (BG <110 mg/dL, n=28), mild hyperglycemia (110 to 150 mg/dL, n=49), and moderate/severe (>150 mg/dL, n=14). The primary endpoint was the occurrence of febrile neutropenia (FN) and documented infection during neutropenia, and the secondary endpoints included organ dysfunction according to the definition used by van den Berghe, acute graft-versus-host disease (GVHD), overall survival, and nonrelapse mortality (NRM).

Results. Although the incidence of FN or documented infections was similar between the three groups, hyperglycemia was significantly associated with an increased risk of organ dysfunction, grade II–IV acute GVHD, and NRM.

Conclusions. While the results suggested an association between the degree of hyperglycemia during neutropenia and an increased risk of posttransplant complications and NRM, the possibility that intensive glucose control improves the outcome after HSCT can only be confirmed in a prospective randomized trial.

Keywords: Allogeneic transplantation, Hyperglycemia, Nonrelapse mortality, Acute graft-versus-host disease.

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Van den Berghe et al. showed with patients nursed in the intensive care unit (ICU) that the rigid control of hyperglycemia with intensive insulin therapy to keep the blood glucose level at 80–110 mg/dL reduced morbidity, including infections, and mortality compared to patients who received standard care maneuvers that maintained the level at <200 mg/dL (*1–3*). Although these results have been confirmed in several subsequent studies (*4–7*), the precise mechanism that underlies this association is unclear. In animal models, it has been shown that insulin itself has a direct inhibitory effect on the inflammation process (*8, 9*). However in human studies, it has been suggested that these benefits could be directly attributed to intense glucose control rather than to any pharmacological activity of administered insulin per se (*3, 4*).

Recipients of allogeneic hematopoietic stem cell transplantation (HSCT) suffer from serious complications including infection, graft-versus-host disease (GVHD) and organ dysfunction. They are also at higher risk of hyperglycemia due to the use of steroids for the treatment of graft-versus-host disease (GVHD), prolonged total parenteral nutrition (TPN), immunosuppressive drugs, and infectious complications (*10, 11*). This makes them susceptible to numerous serious complications, including multiple organ failure (*12–14*). In this study, we evaluated whether hyperglycemia during the cytopenic pe-

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riod after conditioning for HSCT could be a significant risk factor for the subsequent clinical course.

PATIENTS AND METHODS

Patient Characteristics

A cohort of 112 consecutive adult patients who received myeloablative allogeneic HSCT between January 2002 and June 2006 at the National Cancer Center Hospital (Tokyo, Japan) was reviewed retrospectively. Twenty-one patients were excluded due to graft failure, pre-existing infectious diseases or neutropenia before HSCT, and previous allogeneic HSCT. The remaining 91 patients were subjected to further analysis, and their characteristics are listed in Table 1. Their median age was 36 years (range, 18–57 years), and their diagnosis included acute myeloid leukemia (AML, n=41), acute lymphoblastic leukemia (ALL, n=21), non-Hodgkin lymphoma (NHL, n=13), myelodysplastic syndrome (MDS, n=10), and chronic myelogenous leukemia (n=6). Standard-risk patients included those with acute leukemia in first complete remission, chronic leukemia in first chronic phase, MDS in refractory anemia, and NHL in complete remission, and the remaining patients were categorized as high-risk. Forty-six and 45 patients received a graft from a related donor and an unrelated donor, respectively. Stem cell sources included bone marrow (n=46), peripheral blood (n=41), and cord blood cells (n=4). In this study, only two patients were diagnosed as type 2 diabetes mellitus before HSCT, which reflects the low prevalence of this condition in Japan, especially in younger patients who can be the target of allogeneic HSCT with a myeloablative conditioning regimen. These two diabetic patients were included in the moderate and severe hyperglycemia group. None of the patients, including these two patients, had major organ dysfunction or diabetic complications before HSCT. For the transplantation procedure, signed informed consent was obtained according to the Declaration of Helsinki.

Transplantation Procedures

All patients received a myeloablative conditioning regimen that included oral busulfan (BU) plus cyclophosphamide (CY, n=45), CY plus 12 Gy total body irradiation (TBI, n=43) or cytarabine (CA) plus CY plus TBI (n=3: Table 1). GVHD prophylaxis included cyclosporine- (n=62) and tacrolimus-based regimens (n=29), with an additional short course of methotrexate (MTX) in 89 patients. Granulocyte

TABLE 1. Patient characteristics

Variable	Normoglycemia (<110 mg/dl)	Mild hyperglycemia (110–150 mg/dl)	Moderate and severe hyperglycemia (>150 mg/dl)
N	28	49	14
Blood glucose, median mg/dl (range)	104 (81–109)	120 (110–150)	168 (150–211)
Age, median years (range)	31 (21–52)	36 (18–57)	45 (30–57)
<40	20 (71)	32 (65)	4 (29)
≥40	8 (29)	17 (35)	10 (71)
Sex			
Male	9 (32)	34 (69)	8 (57)
Female	19 (68)	15 (31)	6 (43)
Disease risk			
Standard	16 (57)	18 (37)	6 (43)
High	12 (43)	31 (63)	8 (57)
Conditioning			
TBI-containing	11 (39)	26 (53)	9 (64)
Non-TBI-containing	17 (61)	23 (47)	5 (36)
GVHD prophylaxis			
Cyclosporine-based	24 (86)	33 (67)	5 (36)
Tacrolimus-based	4 (14)	16 (33)	9 (74)
Relation to donor			
Related	19 (68)	24 (49)	3 (21)
Unrelated	9 (32)	25 (51)	11 (79)
Stem cell source			
Bone marrow	11 (39)	24 (49)	11 (79)
PBSC	16 (57)	22 (45)	3 (21)
Cord blood	1 (4)	3 (6)	0 (0)
HLA match			
Match	25 (89)	34 (69)	10 (71)
Mismatch	3 (11)	15 (31)	4 (29)

Data are n (%) unless noted.
TBI, total body irradiation; GVHD, graft-versus-host disease; PBSC, peripheral blood stem cells; HLA, human leukocyte antigen.

colony-stimulating factor (G-CSF) was administered in all patients from day +6 after transplantation until engraftment. Most patients received ciprofloxacin (200 mg orally three times daily) for bacterial prophylaxis until neutrophil engraftment. Fluconazole (100 mg once daily) was administered for fungal prophylaxis. Low-dose acyclovir was given for prophylaxis against herpes simplex virus and varicella zoster virus until the cessation of immunosuppressive agents. Prophylaxis against *Pneumocystis jiroveci* infection consisted of trimethoprim-sulfamethoxazole (400 mg of sulfamethoxazole once daily) from the first day of conditioning to day −3 of transplantation, and from day +28 until day +180 or the cessation of immunosuppressive agents. Patients who developed fever during the neutropenic period were treated with cefepime, and additional agents including vancomycin, aminoglycosides and amphotericin B were given as clinically indicated. Neutrophil engraftment was defined as the first of 3 consecutive days after transplantation that the absolute neutrophil count exceeded 0.5×10^9/L.

Grouping of Patients

Patients were categorized according to the mean blood glucose (BG) level in the preengraftment neutropenic period: normoglycemia BG maintained at <110 mg/dL (group 1, n=28), mild hyperglycemia at 110–150 mg/dL (group 2, n=49), and moderate/severe hyperglycemia at >150 mg/dL (group 3, n=14). Blood glucose level was routinely tested in the morning at least three times a week. Daily caloric intake was calculated by dietitian following the chart record.

Outcome Measures

The primary outcome measure was the occurrence of febrile neutropenia (FN) and documented infection including bacteremia, pneumonia and central venous catheter infection in the neutropenic period. Secondary outcome measurements were organ dysfunction in the neutropenic period, acute GVHD, overall survival (OS) and nonrelapse mortality (NRM). Organ dysfunction was defined with reference to van den Berghe (*5–7*) as follows: 1) hypercreatininemia: serum creatinine level ≥2.0 mg/dL or more than twice the baseline; 2) hyperbilirubinemia: serum total bilirubin level ≥2.0 mg/dL; and 3) increased inflammatory markers: serum C-reactive protein (CRP) level ≥15 mg/dL. Acute GVHD was graded by the Consensus Criteria (*15*).

Statistical Analyses

Standard descriptive statistics were used. The Student's *t*-test, chi-square, and Wilcoxon rank-sum tests were used to compare clinical and patient characteristics. Multiple logistic regression analysis was conducted to ascertain odds ratios (ORs) and 95% confidence intervals (CIs). OS was estimated using Kaplan-Meier curves. The cumulative incidences of NRM were estimated based on a Cox regression model for the cause-specific hazards by treating progressive disease or relapse as a competing event. Cox proportional hazard models were used for multivariate analysis of variables on NRM and OS after HCT. Clinical factors that were assessed for their association with NRM and OS included patient age, sex, conditioning regimen (TBI-based vs. non-TBI-based), donor [human leukocyte antigen (HLA)-matched vs. HLA-mismatched, related vs. unrelated], GVHD prophylaxis (cyclosporine-based vs. tacrolimus-based) and disease risk (standard vs. high). Factors with $P<0.10$ in the univariate analyses were subjected to a multivariate analysis. A level of $P<0.05$ was defined as statistically significant. All P values are two-sided. All analyses were performed using SPSS 10.0 statistical software (Chicago, IL).

RESULTS

Patients and Transplantation Characteristics

The median ages of the patients in the normoglycemia, mild hyperglycemia, and moderate/severe hyperglycemia groups were, respectively, 31, 36, and 45 years. The percentages of patients who received graft from an unrelated donor were 32%, 51%, and 79%, and the percentages of patients who received GVHD prophylaxis with tacrolimus were 14%, 33%, and 74%. To clarify the risk factor to be included in moderate and severe hyperglycemia group, logistic analysis was performed, which showed older age and GVHD prophylaxis with tacrolimus were associated with moderate and severe hyperglycemia [$P=0.04$, OR 3.9 (1.1–14.0), and $P=0.01$, OR 5.5 (1.5–20.3), respectively], and there was a trend that patients who received stem cell from unrelated donor were associated with moderate and severe hyperglycemia [$P=0.07$, OR 3.6 (0.9–14.2)]. Multiple logistic analysis showed age more than 40 years old and GVHD prophylaxis with tacrolimus were associated with moderate and severe hyperglycemia [$P=0.042$, OR 4.1 (1.1–15.7), and $P=0.01$, OR 5.8 (1.5–22.1), respectively].

Although in practice we generally keep the parenteral glucose dose relatively low to avoid severe metabolic complications including hyperglycemia and hyperlipidemia during the acute phase of allogeneic HSCT, the possibility that the dose of parenteral nutrition affects the blood glucose level should be explored. We calculated the total caloric intake by combining both oral and parenteral nutrition. Although the mild hyperglycemia group received significantly more parenteral nutrition than the normoglycemia group (group 1 694+322 kcal/day vs. group 2 969+383 kcal/day), overall there was no essential difference in caloric intake between the three groups (1070+303 kcal/day, 1190+393 kcal/day, 1045+530 kcal/day, respectively). The median duration of the follow-up time in surviving patients was 809 days (range, 132-1530 days) in group 1, 369 days (105–1550 days) in group 2, and 587 days (170–774 days) in group 3. Described as hydrocortisone-equivalent dose, the median dose of corticosteroid used during neutropenia was 0 mg (0–1610 mg) in group 1, 100 mg (0–9700 mg) in group 2, and 375 mg (0–2468 mg) in group 3. Statistically more dose of corticosteroid was used in group 2 and group 3, compared with group 1.

Primary Endpoints

The incidence of FN and documented infections is summarized in Table 2. The incidences of FN and documented infections including bacteremia, pneumonia, and central venous catheter infection in groups 1, 2 and 3 were, respectively, 89% and 32% (25%, 4% and 11%), 88% and 20% (16%, 6% and 6%), and 98% and 43% (36%, 14% and 14%). Overall, no statistically significant difference was observed between the three groups in the incidence of infectious episodes, including FN and documented infections.

TABLE 2. Endpoints

Variable	Normoglycemia (<110 mg/dl)	Mild hyperglycemia (110–150 mg/dl)	Moderate and severe hyperglycemia (>150 mg/dl)
N	28	49	14
Febrile neutropenia	23 (89)	43 (88)	13 (98)
Documented infection	9 (32)	10 (20)	6 (43)
Bacteremia	7 (25)	8 (16)	5 (36)
Pneumonia	1 (4)	3 (6)	2 (14)
Central-venous catheter infection	3 (11)	3 (6)	2 (14)
Organ dysfunction			
Hypercreatininemia	1 (4)	4 (8)	4 (29)
Hyperbilirubinemia	3 (11)	11 (22)	6 (43)
Increased inflammatory markers	4 (14)	15 (31)	9 (64)

Data are n (%).
Hypercreatininemia, serum creatinine level ≥2.0 mg/dl or more than twice of baseline; hyperbilirubinemia, serum bilirubin level ≥2.0 mg/dl; increased inflammatory markers, serum C-reactive protein level ≥15 mg/dl.

Secondary Endpoints

The incidence of hypercreatininemia was 4% in group 1, 8% in group 2 and 29% in group 3, as summarized in Table 2, and that in group 3 was significantly higher than those in group 1 (OR 10.8, 95% CI 1.1–108.6; $P=0.018$) and group 2 (OR 4.5, 95% CI 1.0–21.1; $P=0.043$). The incidence of hyperbilirubinemia was, respectively, 11%, 22% and 43%, in the three groups, and that in group 3 was significantly higher than that in group 1 (OR 6.3, 95% CI 1.3–30.9; $P=0.017$). The incidence of increased inflammatory markers was, respectively, 14%, 31% and 64%, and that in group 3 was significantly higher than those in group 1 (OR 10.8, 95% CI 2.4–49.5; $P<0.001$) and group 2 (OR 4.1, 95% CI 1.2–14.3; $P=0.022$). Multiple logistic regression analysis showed that the degree of hyperglycemia was associated with hypercreatininemia, hyperbilirubinemia, and increased inflammatory markers (Table 3).

TABLE 3. Multiple logistic regression analysis for organ dysfunction and multiple variate analysis for acute GVHD, nonrelapse mortality, and overall survival

Outcomes and variables	Odds/hazard ratio	95% CI	*P* value
Multiple logistic regression analysis			
Hypercreatininemia			
Hyperglycemia	5.2	1.1–24.6	0.039
Hyperbilirubinemia			
Hyperglycemia	4.9	1.6–14.9	0.005
Increased inflammatory markers			
Hyperglycemia	6.7	2.2–20.3	0.001
Tacrolimus-based	6.9	1.6–30.5	0.011
Multivariate analysis (Cox-proportional hazard model)			
Acute GVHD			
Hyperglycemia	2.3	1.2–4.3	0.013
Disease risk (high)	2.3	1.0–5.1	0.047
HLA mismatch	2.8	1.3–5.9	0.009
Nonrelapse mortality			
Hyperglycemia	2.9	1.2–6.6	0.013
Disease risk (high)	2.7	0.9–8.7	0.091
Overall survival			
Hyperglycemia	2.0	1.1–3.6	0.019
TBI-containing	2.3	1.1–5.0	0.035
Disease risk (high)	1.9	0.9–4.1	0.10

Odds ratios are presented for multiple logistic regression analysis; hazard ratios are presented for multivariate analysis.
GVHD, graft versus host disease; TBI, total body irradiation.

The cumulative incidence of grade II–IV acute GVHD is shown in Figure 1. The degree of hyperglycemia was associated with a higher incidence of grade II–IV acute GVHD

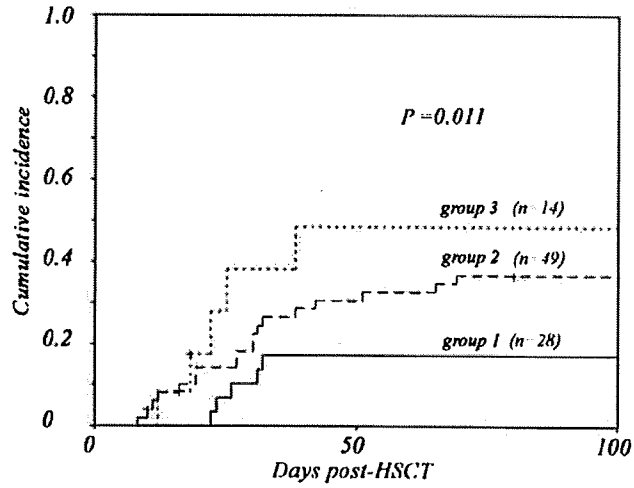

FIGURE 1. Cumulative incidence of acute GVHD grade II–IV stratified according to the mean glucose level during neutropenia. Group 1 included patients with normoglycemia, group 2 included patients with mild hyperglycemia, and group 3 included patients with moderate and severe hyperglycemia.

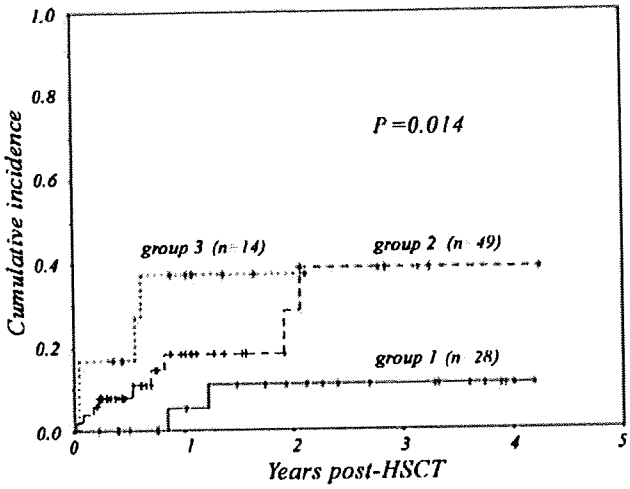

FIGURE 2. Cumulative incidence of treatment-related mortality stratified according to the mean glucose level during neutropenia.

(P=0.002). A Cox proportional hazard model showed that hyperglycemia, high-risk underlying disease, and HLA mismatch were risk factors for grade II-IV acute GVHD (Table 3).

The cumulative incidence of NRM was, respectively, 5%, 17%, and 35% at 1 year, and was significantly related to the degree of hyperglycemia (P=0.014; Fig. 2). The probability of OS was, respectively, 88%, 70%, and 56%, and was significantly associated with hyperglycemia (P=0.008; Fig. 3). A Cox proportional hazard model showed that the degree of hyperglycemia was associated with NRM and OS (Table 3).

DISCUSSION

In this study, we evaluated whether hyperglycemia during the cytopenic period after conditioning for HSCT could be a significant risk factor for the subsequent clinical course. Infectious diseases remain a major cause of morbidity and mortality in patients who receive HSCT, and we speculated that this might be exaggerated in the presence of hyperglycemia. Alternatively, hyperglycemia can be caused by infectious diseases and also aggravates infectious diseases to lead to a vicious cycle, with resultant morbidities that include organ dysfunction and mortality. Theoretically, strict glucose control should prevent this vicious cycle and help to reduce morbidity and mortality in patients after HSCT, as shown previously in ICU settings (*1, 2*). However, in this study the incidences of FN and documented infections were not different among the three groups. On the other hand, we found that hyperglycemia was associated with organ dysfunction and increased inflammatory markers, which was consistent with previous reports that demonstrated the impact of hyperglycemia on clinical outcomes of patients suffering from nonhematological diseases (*1–3, 12–14*). Additionally, a multivariate analysis showed that hyperglycemia was a risk factor for acute GVHD.

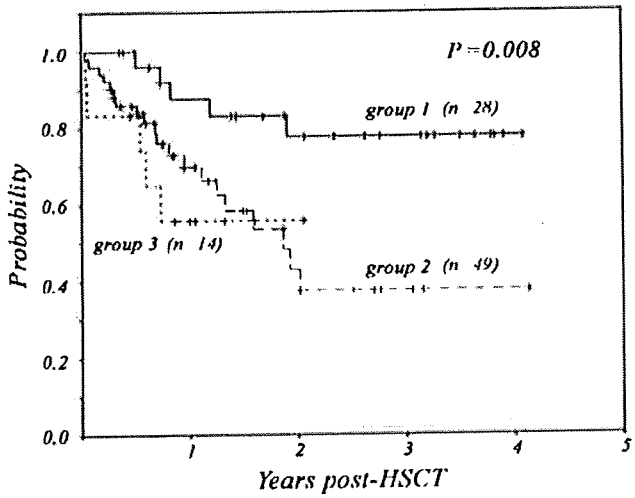

FIGURE 3. Overall survival stratified according to the mean glucose level during neutropenia.

The reason for the association between early hyperglycemia and late complications needs to be clarified. The increase in the levels of circulating cytokines due to hyperglycemia may further aggravate hyperglycemia itself (*16–21*). Therefore, this condition which occurs during the critical period of neutropenia before engraftment may influence the afferent phase of acute GVHD, as suggested by Ferrara et al. Elevated cytokine levels during the afferent phase then lead to subsequent acute GVHD in the effector phase (*22, 23*). Teshima et al. reported that the effector phase of acute GVHD is not antigen-specific and inflammatory cytokines mediate target destruction (*24*), and other reports have shown that inflammatory cytokines were required in acute GVHD and these molecules can cause tissue damage (*25–27*). With these reports in mind, it is reasonable to speculate that the aggravated production of inflammatory cytokines by hyperglycemia may be a risk factor in the pathogenesis of acute GVHD and organ dysfunction.

This study has several limitations, including heterogeneous patient populations and a retrospective nature. First, hyperglycemia can be caused by infection itself and it has been previously shown that the level of hyperglycemia was correlated with the severity of illness (*4*). In this retrospective study, we could not confirm whether hyperglycemia directly influenced organ dysfunction or increased inflammatory markers. Furthermore, statistically more corticosteroid was used in the group of moderate and severe hyperglycemia, and statistically more parenteral nutrition was used in the group of mild hyperglycemia. However, the observation that hyperglycemia and the severity of illness were independently associated with a worse prognosis has been well confirmed in the ICU setting (*4*), and several prospective studies have shown that intensive glucose control reduced both morbidity and mortality (*1, 2*). Considering these findings, we suggest that our data still support the possibility that the degree of hyperglycemia was associated with morbidity and mortality in the allogeneic HSCT setting. Second, we must consider that the patients who developed moderate and severe hyperglycemia included older patients, those who received more unrelated grafts, and those who received tacrolimus compared to other groups. In terms of immunosuppressive drugs, tacrolimus has recently become a preferred immunosuppressive drug for GVHD prophylaxis in unrelated or HLA-mismatched HSCT, based on the results of two Japanese studies, which showed that, compared to cyclosporine, tacrolimus was associated with a lower incidence of acute GVHD and better overall survival, which were similar to those in related HSCT, even

after HSCT with alternative donors, including unrelated donors (*28, 29*). Therefore, the effect of unrelated graft and tacrolimus on the incidence of acute GVHD and NRM might not be significant in this study.

The effects of tacrolimus on hyperglycemia, hyperbilirubinemia, and hypercreatininemia need to be clarified. It is well known that hyperglycemia occurs more often in patients receiving tacrolimus than in those receiving cyclosporine (*30–32*). In the present study, patients receiving tacrolimus were more likely to have moderate to severe hyperglycemia. However, the association of hyperbilirubinemia with tacrolimus has not been previously reported and two other studies (*33, 34*) showed that cyclosporine was more likely to cause hyperbilirubinemia than tacrolimus after allogeneic HSCT or kidney transplantation. Although the relative nephrotoxicity attributed to tacrolimus compared to cyclosporine has been controversial (*30, 33, 35*), studies that have reported such nephrotoxicity used a higher target tacrolimus level (>20 ng/ml) (*30, 35*). On the other hand, it has been reported that the use of lower levels of tacrolimus (10–15 ng/ml in our hospital) was associated with reduced complications in allogeneic HSCT (*36, 37*), with no difference in the incidence of hypercreatininemia compared to cyclosporine (*33*). Based on a consideration of all of these results, we think that tacrolimus might not be the direct cause of hypercreatininemia in this study. Finally, due to the nature of this retrospective study, during the period evaluated we did not apply any consistent protocol for glucose control and nutritional support, although we tried to avoid severe hyperglycemia (BG ≥200 mg/dl), which certainly biases the interpretation of the data, although it has been reported that the overall glucose level, rather than the dose of insulin administered, directly influenced the outcome of patients (*3*).

Even with these limitations, we believe that our observation is still of value in considering the clinical impact of the strict control of hyperglycemia during the early phase of HSCT. To confirm our preliminary observation, a prospective pilot study is underway to assess the effect of intensive glucose control after HSCT. If this pilot study shows a beneficial effect of intensive glucose control, a prospective randomized trial would be warranted to confirm the possibility that intensive glucose control improves the outcome after HSCT. Additionally, in this ongoing pilot study, we evaluate the diurnal blood glucose and insulin levels, including postprandial levels, to detect hyperglycemia more precisely before transplantation since the level of HgA1c is affected by both the blood glucose level and the turnover rate of red blood cells, and would not precisely correlate with the true mean blood glucose level in patients who received courses of blood transfusion for anemia.

In conclusion, the association of the degree of hyperglycemia during neutropenia and an increased risk of post-transplant complications and NRM was suggested, but the possibility that intensive glucose control improves the outcome after HSCT would only be confirmed in a prospective randomized trial.

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T-Cell Large Granular Lymphocyte Leukemia of Donor Origin After Cord Blood Transplantation

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Abstract

We report the first case of T-cell large granular lymphocyte leukemia of donor origin after a second cord blood transplantation for acute myeloid leukemia, and review the literature regarding rare cases of T-cell–origin posttransplantation lymphoproliferative disorders.

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Key words: Bone marrow, Epstein-Barr virus, Polymerase chain reaction, Posttransplantation lymphoproliferative disorders, T-cell receptor

Introduction

T-cell large granular lymphocyte leukemia (LGL; LGLL) is characterized by the monoclonal proliferation of $CD3^+$, and $CD8^+$ LGLs, with abundant cytoplasm and fine or coarse azurophilic granules.[1,2] Reactive expansion of LGL in the peripheral blood has been occasionally reported during viral infection and in recovery phase of allogeneic hematopoietic stem cell transplantation (HSCT).[3,4]

Posttransplantation lymphoproliferative disorder (PTLD) is a characteristic lymphoid proliferation or the development of lymphoma in a setting of decreased T-cell immune surveillance, typically in recipients of solid organ transplantation or allogeneic HSCT. Most reported cases of PTLD are of B-cell origin, in association with Epstein-Barr virus (EBV) infection, which leads to monoclonal or, less frequently, polyclonal proliferation of B cells. Most of the rare cases of T-cell PTLD were reported after solid organ transplantation, with very rare cases after allogeneic HSCT.

In this report, we describe the unique clinical and laboratory findings of a patient with γδ T-cell LGLL of cord donor origin after a second cord blood transplantation for acute myeloid leukemia.

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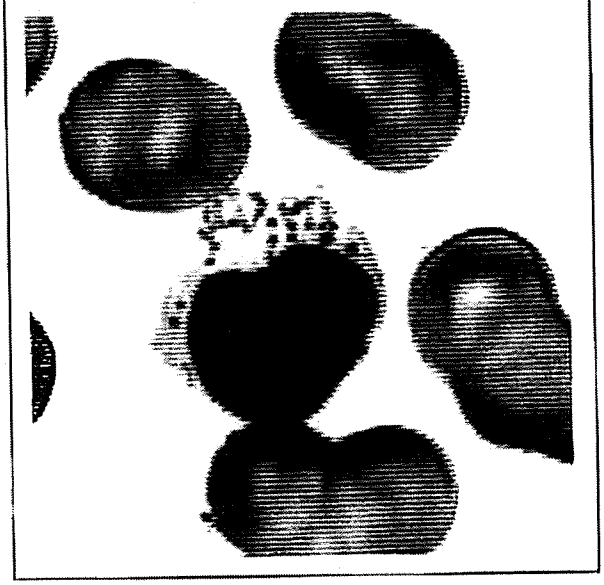
Case Report

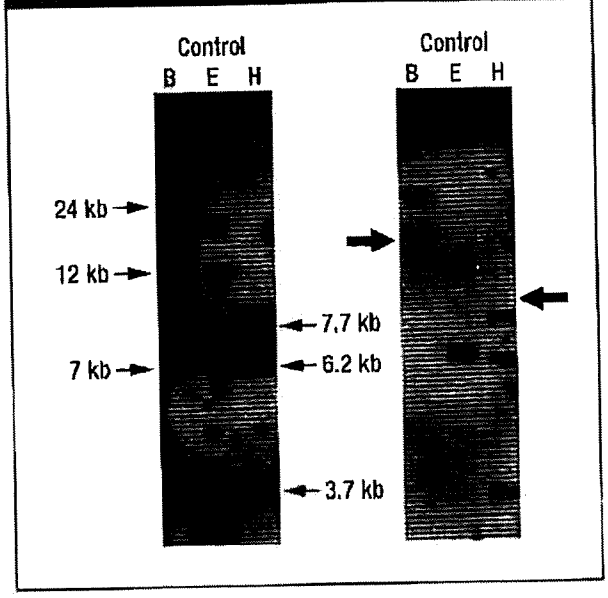
A 58-year-old Japanese man with acute myeloid leukemia (French-American-British classification; M2) in second complete remission received allogeneic HSCT from an unrelated female cord blood donor. The conditioning regimen consisted of total body irradiation of 12 Gy in 6 fractions from day −6 to −4, and cyclophosphamide 60 mg/kg once daily intravenously on days −3 to −2 (total dose, 120 mg/kg). He received human leukocyte antigen–loci mismatched (2 by serology and 2 by DNA typing) unrelated cord blood, which contained 3.03×10^7 nucleated cells/kg in January 2003. Cyclosporine and short-term methotrexate were used as graft-versus-host disease prophylaxis. However, hematologic recovery was not observed up to day 40, and we concluded that this was a case of primary graft failure without leukemia relapse because the results of interphase fluorescence in situ hybridization analysis on days 23, 30, and 37 on bone marrow (BM) samples were negative. Because his condition remained good, we planned a second cord blood transplantation with a reduced-intensity regimen, which consisted of fludarabine 30 mg/kg once daily intravenously from days −8 to −3 (total dose 180 mg/kg), busulfan 4 mg/kg orally on days −6 and −5 (total dose 8 mg/kg), and total body irradiation of 4 Gy in 1 fraction on day −1. Cyclosporine and mycophenolate mofetil 15 mg/kg twice daily were administered. On day 51 of the initial transplantation in March 2003, human leukocyte antigen–loci mismatched (2 by serology and 3 by DNA typing) male cord blood, containing 2.6×10^7/kg nucleated cells, was infused. Neutrophil engraftment was observed by day 33 after second transplantation. Acute and chronic graft-versus-host disease did not develop, and cyclosporine was tapered off in November 2003.

Figure 1 T-Cell Large Granular Lymphocyte Leukemia Stained with May-Giemsa on the Peripheral Blood Smear



The predominant cells were typical of LGLs with abundant cytoplasm and fine or coarse azurophilic granules.
Hematoxylin and eosin stain. original magnification ×1000

Figure 2 Southern Blots of T-Cell Receptor β-Chain Gene Rearrangements



DNA from BM of this patient was hybridized with a TCR Cβ1 probe
Arrows indicate rearranged bands
Abbreviations: B = Bam HI; E = Eco R; H = Hind III

In February 2004, 10 months after the second cord blood transplantation, he developed anorexia, abdominal distention with fluid accumulation, and edema in the lower extremities. A computed tomography scan showed gross ascites and mild pleural effusion but no sign of enlarged lymph nodes or hepatosplenomegaly. The peripheral white blood cell count was 10,300/μL (10.3 × 10^9/L), and 30% of the cells had a morphology of medium to large lymphocytes with abundant azurophilic granules in the cytoplasm, as shown in Figure 1. The hemoglobin level was 8.8 g/dL (88 g/L), and the platelet count was 192 × 10^3/μL (1.92 × 10^9/L).

A retrospective review of the peripheral blood smears disclosed that the appearance of LGL coincided with the tapering off of immunosuppression 3 months before the admission.

Flow cytometry examination of the peripheral blood mononuclear cells showed a homogeneous population of T-cell LGLs positive for CD2, CD3, CD8, CD56, and T-cell receptor (TCR)–γδ, but negative for CD4 and TCR-αβ. The BM biopsy specimen histologically showed 10% of hypocellular gelatinous marrow with diffuse infiltration of medium to large lymphoid cells. Immunoperoxidase studies on sections of BM showed strong expression of T-cell–restricted intracellular antigen-1, partially positive staining of CD8 and granzyme B, but no expression of CD3 or CD20. Southern blot analysis of the BM cells revealed a clonal rearrangement of the TCR-β chain, as shown in Figure 2 and TCR-δ chain (data not shown).

Abdominal paracentesis was performed with milky chylous fluid, and a flow cytometry examination showed results similar to those in the peripheral blood. Multiprimer-based polymerase chain reaction (PCR) analysis of ascitic cells also showed clonal rearrangement of the TCR-δ chain, as shown in Figure 3. The primer sets were used in the following locations: Vδ1, 5'-AAA CTG GTC GCT ATT CTG TC-3'; Vδ2A, 5'-GCA CCA TCA GAG AGA GAT GA-3'; Jδ, 5'- TGG TTC CAC AGT CAC ACG GG-3'; Dδ3B, 5'-TTG TAG CAC CGT GCG TAT CC-3'. The amplified 200 base-pair PCR products of the TCR-δ chain were then cloned into the pCR-TOPO vector. The DNA sequences of 3 clones amplified by vectors were identical and had high homology to TCR-δ chain including a 197 base-pair sequence (data not shown). This sequence also involved the forward and reverse primers Vδ1 and Jδ, respectively, described previously.

The results of all of the previously mentioned studies indicated the clonal expansion of T cells compatible with a diagnosis of T-cell LGLL with γδ T-cell phenotype involving peripheral blood, BM, and ascites.

Donor-recipient DNA chimerism was analyzed by comparing the short tandem repeat findings for the donor blood sample and pretransplantation recipient samples. Eleven short tandem repeat loci were analyzed by PCR using an AmpFlSTR SGM Plus® kit. The peripheral blood sample (containing 30% T-LGL) and the second cord blood sample showed the same peaks at the locus (D16S539), as shown in Figure 4. These results further confirmed that the expanded γδ T-LGL cells were exclusively of second cord blood transplantation donor origin.

Serologic examination showed no evidence of viral infection. Real-time PCR analysis revealed a high load of EBV (7.9 × 10^3 copies/10^6 cells). However, in situ hybridization studies of BM cells did not reveal EBV-encoded small RNA, and Southern blot analysis of BM cells also showed no band for