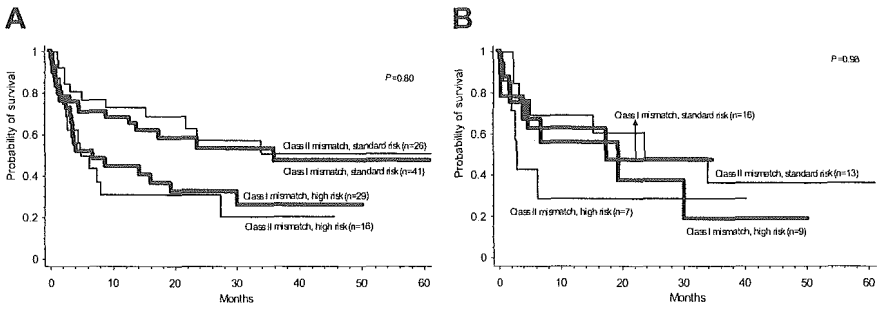

Figure 3. Overall survival based on mismatch locus. Overall survival after serologically (A) and genotypically (B) one-locus–mismatched HSCT in class I versus class II mismatch, stratified by the disease status. *P* values for class I versus class II mismatch are shown.

blood stem cell (PBSC) grafts were included in the PBSC group. This study was approved by the Committee for Nationwide Survey Data Management of the Japan Society for Hematopoietic Cell Transplantation.

Results

Characteristics of the patients

The numbers of patients who received a graft from a serologically HLA-matched, one-locus–mismatched, and 2- or 3-loci–mismatched family donor were 2805, 112, and 30, respectively (Table 1). The HLA-mismatched group included significantly higher proportions of patients with high-risk disease and those who received PBSCs ($P < .0001$ for both comparisons). TBI-based conditioning regimens were preferentially used in HLA-mismatched group ($P = .02$) and high-risk group ($P < .0001$).

We determined the number of genotypically mismatched loci in 2845 patients by the method described (see "Histocompatibility") and the results are shown at the bottom of Table 1. An additional genotype mismatch was detected in 8 patients.

HLA-mismatch vector

Among the 112 patients who received a serologically one-locus–mismatched graft, 70 had a bidirectional mismatch, whereas 15 and 27 had a mismatch only in the GVH and HVG vectors, respectively. To evaluate the influence of mismatch in the GVH vector, we compared survival and the incidence of acute GVHD among patients who received serologically one-locus–mismatched grafts for those with and without mismatch in the GVH vector. Although the survival rate in the 2 groups was almost equivalent ($P = .72$), the incidence of grade III to IV acute GVHD was significantly higher in patients who underwent HSCT with a mismatch in the GVH vector ($P = .02$; Figure 1). Therefore, we used the overall number of mismatches in the survival analysis, whereas we used the number of mismatches in the GVH vector to compare the incidence of acute GVHD.

Engraftment failure

The incidence of engraftment failure was significantly higher in pairs with an HLA mismatch between the donor and recipient (2.4% in a serologically matched cohort versus 6.3% in a mismatched cohort; $P = .01$) and this difference was seen regardless of whether the HLA mismatch was analyzed at the serologic level or the DNA level and whether the mismatch was considered only in the HVG vector or in both vectors. Logistic regression analysis identified 2 independent risk factors for engraftment failure: HLA mismatch in the HVG vector (odds ratio, 2.28; 95% CI, 1.09-4.75; $P = .028$) and high-risk disease (odds ratio, 3.77; 95% CI, 2.36-6.02; $P < .0001$).

Survival

Overall survival in patients who underwent HSCT for standard-risk and high-risk diseases, grouped by the number of serologic HLA mismatches, is shown in Figure 2, panels A and B, respectively. A higher number of serologic HLA mismatches appeared to adversely affect survival. Among the potential confounding factors, higher age (≥ 40 years old), high-risk disease, and HLA mismatch were identified as independent predictive factors for shorter survival (Table 2). However, the impact of HLA mismatch on survival was smaller in high-risk patients and there was no statistically significant difference in survival between HLA-matched HSCT and one-locus–mismatched HSCT for high-risk disease ($P = .24$).

We further analyzed the impact of genotypic HLA mismatch to exclude the influence of an unrecognized mismatch at the DNA level. As shown in Figure 2C-D, we observed a similar tendency toward a smaller impact of HLA mismatch on survival in patients with a high-risk disease, although the presence of genotypic HLA mismatch, along with an older age (≥ 40 years old), and high-risk disease, was identified as an independent risk factor for shorter survival (Table 2).

We compared the influence of class I versus class II mismatch on survival after serologically one-locus–mismatched HSCT, but the survival curves after HSCT with a class I mismatch and those

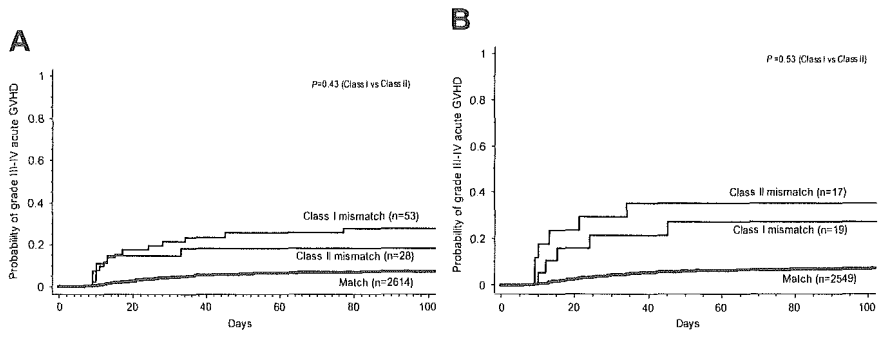

Figure 4. Incidence of grade III to IV acute GVHD. Cumulative incidence of grade III to IV acute GVHD after serologically (A) and genotypically (B) HLA-matched or one-locus–mismatched HSCT. *P* values for class I versus class II mismatch are shown.

Table 3. Results of proportional hazards modeling for the development of grade III to IV acute GVHD

	Relative risk (95% CI)	P
Serologic matching		
Age		
Younger than 40 y	1.00	.027
40 y and older	1.36 (1.04-1.79)	
Disease		
Standard risk	1.00	.015
High risk	1.43 (1.07-1.92)	
Sex		
Female	1.00	< .0001
Male	1.86 (1.37-2.53)	
Stem cell		
BMT	1.00	< .0001
PBSCT	2.24 (1.63-3.08)	
HLA		
GVH match	1.00	< .0001
GVH mismatch	2.67 (1.65-4.33)	
Genotypic matching		
Age		
Younger than 40 y	1.00	.041
40 y and older	1.34 (1.01-1.78)	
Disease		
Standard risk	1.00	.024
High risk	1.41 (1.01-1.90)	
Sex		
Female	1.00	.0002
Male	1.81 (1.33-2.48)	
Stem cell		
BMT	1.00	< .0001
PBSCT	2.47 (1.79-3.41)	
HLA		
GVH match	1.00	< .0001
GVH mismatch	5.41 (2.93-10.0)	

Two- or 3-loci–mismatched transplants were excluded. BMT indicates bone marrow transplantation; PBSCT, peripheral blood stem cell transplantation.

after HSCT with a class II mismatch were superimposed (Figure 3A; $P = .80$). Furthermore, in an analysis at the DNA level, there was no statistically significant difference in survival after genotypically one-locus–mismatched HSCT between patients who received class I mismatched graft and those who received class II mismatched graft (Figure 3B; $P = .98$).

Acute and chronic GVHD

The incidence of grade III to IV acute GVHD after serologically one-locus–mismatched HSCT was 31%, which was significantly higher than that after serologically matched HSCT (9%; $P < .0001$). In addition, more than half the patients who developed grade III to IV acute GVHD did so between 10 and 20 days after HSCT (Figure 4A). Male sex, older age (≥40 years old), high-risk disease, the use of PBSCs, and the presence of HLA mismatch in the GVH vector were identified as independent risk factors for the development of grade III to IV acute GVHD (Table 3). The impact of one-locus mismatch on the incidence of grade III to IV acute GVHD was almost equivalent in BM transplantation (relative risk, 3.98; 95% CI, 2.25-7.04; $P < .0001$) and PBSC transplantation (relative risk, 2.29; 95% CI, 0.97-5.43; $P = .059$).

We compared the influence of class I versus class II mismatch on the incidence of acute GVHD after serologically one-locus–mismatched HSCT and did not observe a significant difference between the groups ($P = .23$; Figure 4A). In an analysis of genotypic mismatch, the incidence of acute GVHD was almost the same between transplants with class I mismatch and those with class II mismatch ($P = .70$; Figure 4B).

The incidence of chronic GVHD in patients who received a serologically one-locus–mismatched graft was higher than that in patients who underwent HLA-matched HSCT, but this difference was not statistically significant (60% versus 47%; $P = .11$). Multivariate analysis revealed that male sex, higher age (≥ 40 years old), high-risk disease, and the use of PBSCs were independent risk factors for the development of chronic GVHD.

Relapse

Cumulative incidence of relapse was compared between HSCT with versus without a serologic mismatch in the GVH vector. It was 22% at 5 years after matched HSCT for standard-risk diseases, which was not significantly different from that after one-locus–mismatched HSCT (15%; $P = .25$; Figure 5A). In contrast, the incidence of relapse was dramatically decreased after one-locus–mismatched HSCT compared to matched HSCT for high-risk diseases (19% versus 47%; $P = .004$; Figure 5B).

Comparison between one-locus–mismatched related HSCT and matched unrelated HSCT

For patients with a high-risk disease, it appeared unnecessary to search a matched unrelated donor if the patient has a one-locus–mismatched family donor. However, for patients with a standard-risk disease, whether we should choose a one-locus–mismatched family donor or a matched unrelated donor is problematic. Therefore, we compared survival after matched related HSCT, one-locus–mismatched related HSCT, and HLA-matched unrelated HSCT. We excluded HSCT that was performed within 180 days after the diagnosis of underlying disease because the interval from diagnosis to HSCT was more than 180 days in 987 of 1002 unrelated HSCTs. Another difference in the characteristics of the patients between one-locus–mismatched group and matched unrelated group was the proportion of patients of older age (40% versus 25%; $P = .0007$). As shown in Figure 6, survival curves of one-locus–mismatched related HSCT and unrelated HSCT were superimposed both among standard-risk and high-risk patients, although the incidence of

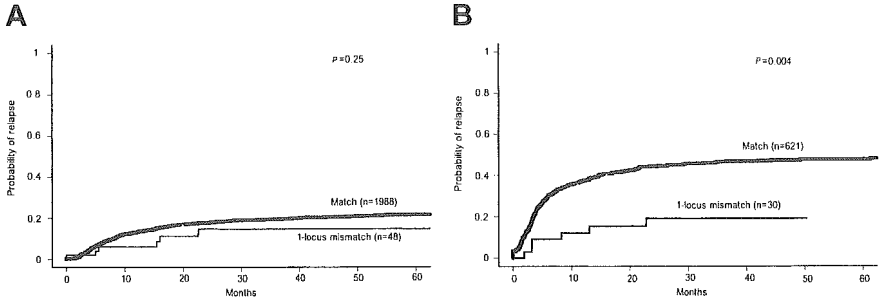

Figure 5. Cumulative incidence of relapse after serologically HLA-matched or one-locus–mismatched HSCT. (A) Standard-risk disease. (B) High-risk disease.

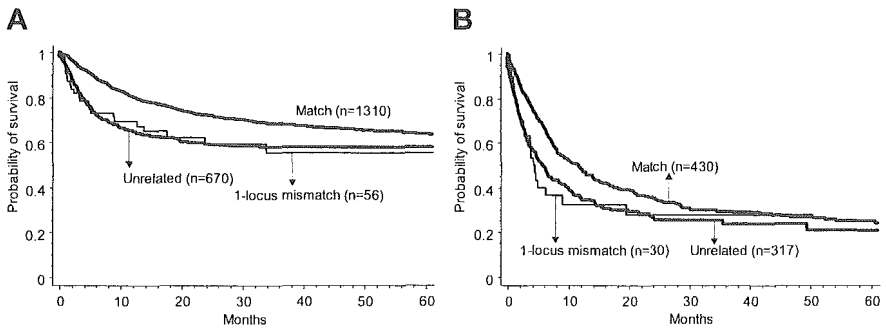

Figure 6. Overall survival after transplantation grouped according to the type of donor and according to the disease status. (A) Standard-risk disease. (B) High-risk disease.

grade III to IV acute GVHD was significantly higher after one-locus–mismatched related HSCT (30% versus 16%; $P = .0013$). These findings did not change when we compared these curves without excluding patients who underwent HSCT that was performed within 180 days after the diagnosis of underlying disease (data not shown). In an analysis of genotypic mismatch, the 2 curves were also superimposed (data not shown).

We performed a multivariate analysis using proportional hazard modeling to adjust the difference between one-locus–mismatched HSCT and matched unrelated HSCT. Higher age (relative risk, 1.61; 95% CI, 1.34-1.94; $P < .0001$) and high-risk disease (relative risk, 2.48; 95% CI, 2.08-2.96; $P < .0001$) were identified as independent risk factors for shorter survival. Whether the use of a one-locus–mismatched related donor or a matched unrelated donor did not affect survival even adjusted for these factors (relative risk, 0.97; 95% CI, 0.71-1.20; $P = .84$).

Discussion

This study analyzed the outcome of HSCT from family donors over the last decade. Recent advances in genomic typing enabled us to evaluate the true influence of a single HLA mismatch. Previous studies did not use genotypic matching and therefore may have overlooked mismatches at the DNA level. Other advantages of this study are the exclusion of ex vivo manipulation of graft and the use of registry data from a homogenous population. These features allowed us to clearly evaluate the influence of HLA-mismatch on the incidence of GVHD and the probability of survival.

Higher age, high-risk disease, and the presence of HLA mismatch were identified as independent risk factors for both shorter survival and the development of grade III to IV acute GVHD. The adverse influence of HLA mismatch on survival was pronounced in patients with standard-risk disease. It is possible that the increased risk of acute GVHD was counterbalanced by a decrease in relapse in patients with high-risk disease, whereas the increased risk of transplant-related mortality did not balance the change in the relapse rate in standard-risk patients, because the risk of relapse is low in such patients.

Whereas the report from IBMTR showed a trend similar to ours, the Seattle group reported equivalent survival in HLA-matched and one-locus–mismatched HSCT, even in standard-risk patients.[4,6,10] This discrepancy may have been due to the difference in the method used for HLA matching.[1] The one-locus–mismatched group in the IBMTR study may have included a greater number of patients with another genotypic mismatch that could not be detected by serologic typing, compared to the Seattle study. However, in this study, we used genotypic matching, and thus it is very unlikely that patients in our one-locus–mismatched group in Figure 2C-D had another genotypic mismatch. Even this "true one-locus mismatch" was shown to adversely affect survival in standard-risk patients. The difference in the impact of HLA-mismatch among studies may be due to recent improvements in the outcome of standard-risk HSCT. The probability of survival at 5 years after HLA-matched standard-risk transplantation was approximately 40%, 60%, and 60% in the Seattle study, the IBMTR study, and this study, respectively, with a similar definition of standard-risk disease.[4,10] The major difference was that the Seattle study included transplantations that were performed between 1975 and 1986, whereas the IBMTR study and this study included those performed between 1985 and 1991, and between 1991 and 2000, respectively. The outcome of HLA-matched transplantation in standard-risk patients might have been improved by advances in supportive treatments and possibly by more ready application of HSCT in standard-risk patients. On the other hand, the outcome of HLA-matched transplantation in high-risk patients has remained fairly constant at approximately 20% survival at 5 years in all 3 studies.

Our comparison of the impact of class I versus class II mismatch on the incidence of acute GVHD and survival is interesting because genotypic class I mismatch was a stronger risk factor than genotypic class II mismatch for the development of grade III to IV acute GVHD in serologically matched unrelated BM transplantation in Japan.[15] However, there was no difference in the outcome of serologically class I mismatched HSCT versus serologically class II mismatched HSCT in this population. Although there were only a small number of patients with complete genotypic matching, whether the mismatch is in class I or class II appeared to be unimportant in one-locus–mismatched HSCT from family members.

The use of PBSCs as a graft was identified as an independent risk factor for the development of grade III to IV acute GVHD. However, in several randomized controlled trials that compared PBSC transplantation and BM transplantation from HLA-identical siblings, the incidence of acute GVHD did not significantly differ, except in a study by the European Group for Blood and Marrow Transplantation.[20-25] In Japan, PBSCs tended to be used in patients with infection or low performance status until allogeneic PBSC transplantation became covered by health insurance in April 2000. Therefore, there might be biases that could not be detected in this dataset. We need a randomized controlled trial to address this issue in our own country.

After we found that the TBI-based regimens were preferentially used in HLA-mismatched group and high-risk patients, we evaluated the impact of the type of a conditioning regimen. However, the difference in the conditioning regimen did not affect any outcome measures. Conditioning regimens including antithymocyte globulin were used in 6 and 3 patients who underwent HLA-matched and

one-locus–mismatched HSCT, respectively. However, exclusion of these 9 patients did not influence the outcome of this study (data not shown).

In conclusion, in high-risk patients, there appears to be no need to search for a matched unrelated donor when a one-locus–mismatched family donor is available, regardless of whether the mismatch is class I or class II, because we can expect an outcome similar to that after HSCT from an HLA-identical sibling. However, in standard-risk patients, the indications for allogeneic HSCT based on randomized controlled trials using HLA-identical sibling donors should not be extended to one-locus–mismatched HSCT, because there was a statistically significant difference in survival between HLA-matched and one-locus–mismatched HSCT. Therefore, we should reconsider the indication of HSCT, although there is no need to search for an unrelated donor when a one-locus–mismatched family donor is available. The outcome of 2- or 3-loci–mismatched HSCT without T-cell depletion was extremely poor.

Acknowledgment

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Unrelated bone marrow transplantation for non-Hodgkin lymphoma: a study from the Japan Marrow Donor Program

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There is little information available regarding the outcome of unrelated bone marrow transplantation (BMT) for non-Hodgkin lymphoma (NHL). Therefore, we retrospectively analyzed the data of 124 patients who underwent unrelated BMT through the Japan Marrow Donor Program (JMDP) between July 1992 and August 2001. The overall survival (OS), progression-free survival (PFS), cumulative incidences of disease progression, and nonprogression mortality at 3 years after BMT were 49.7%, 42.6%, 24.5%, and 32.9%, respectively, with a median follow-up duration of 565 days among survivors. The incidence of grades II-IV acute graft-versus-host disease (GVHD) was 40.9%. Recipient age, previous history of autologous transplantation, and chemosensitivity at transplantation were independent prognostic factors for OS and PFS. The development of grades II-IV acute GVHD was associated with lower incidence of disease progression after transplantation, which suggested the existence of a graft versus lymphoma effect. Unrelated BMT should be considered as a treatment option for patients with high-risk NHL without an HLA-matched related donor. (Blood. 2004;103:1955-1960)

Introduction

Hematopoietic stem cell transplantation for non-Hodgkin lymphoma (NHL) has been mainly performed using an autologous graft, because the incidence of treatment-related mortality after allogeneic transplantation is as high as 57%.[1] However, relapse is a frequent cause of treatment failure after autologous transplantation.[2,3] The lower relapse rate after allogeneic transplantation and the recent development of supportive treatments to decrease the risk of treatment-related mortality have facilitated the use of allogeneic transplantation for NHL.[1] However, an HLA-matched sibling is available for less than half of the patients. Transplantation from an unrelated donor is a possible alternative for patients who do not have a suitable related donor. To date, however, little information is available regarding the outcome of allogeneic transplantation from an unrelated donor for NHL. Therefore, we retrospectively analyzed the outcome of unrelated bone marrow transplantation for NHL using the database of the Japan Marrow Donor Program (JMDP). The purpose of this study was to elucidate the feasibility of unrelated bone marrow transplantation for NHL and to evaluate the impact of a potential graft-versus-lymphoma effect.

Patients and methods

Patients and transplantation procedure

From July 1992 to August 2001, 124 patients with non-Hodgkin lymphoma (NHL) underwent bone marrow transplantation from a serologically HLA-A, -B, and -DR matched unrelated donor identified through the Japan Marrow Donor Program (JMDP). The application of unrelated transplantation was decided at each center. Fourteen of 19 patients who underwent unrelated transplantation in the first complete remission (CR1) had high-grade lymphoma.

Transplantation was performed according to the protocol of each center, and therefore the conditioning regimen and graft-versus-host disease (GVHD) prophylaxis varied among patients (Table 1). However, 90% of the patients received a total body irradiation (TBI)–containing conditioning regimen. Prophylaxis against GVHD was performed with cyclosporine A or tacrolimus combined with methotrexate with or without corticosteroid in all but one patient. At transplantation, 60 patients were in complete remission (CR) and 60 were not (non-CR). Among the 43 patients whose CR status was reported in detail, 19, 18, 5, and 1 were in CR1, CR2, CR3, and CR4, respectively. Seventy-six patients had chemosensitive disease at transplantation, whereas 33 patients had chemoresistant disease. In this study we defined patients who achieved CR or partial remission (PR) before transplantation as chemosensitive, and patients with responses less than PR were defined as chemoresistant in the same way as previous studies.[4,5] Before unrelated donor transplantation, 18 of 101 patients had undergone high-dose therapy and autologous stem cell transplantation (HDT/ASCT). Information regarding previous treatments except for HDT/ASCT or the results of genomic typing were not available in the dataset.

Histology

The JMDP requested the histologic subtype of NHL according to a unique classification system that was a slight modification of the Working Formulation.[6] However, the respective centers used different classification systems, such as the Working Formulation, Kiel,[7] Lymphoma Study Group

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A complete list of the centers in Japan that participated in the bone marrow transplantations for non-Hodgkin lymphoma facilitated by the Japan Marrow Donor Program (JMDP) appears in the "Appendix."

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Table 1. Patient characteristics

Characteristic	Value
Sex, n, M/F	78/46
Median age at transplantation, y (range)	29 (1-59)
Median interval from diagnosis to transplantation, d (range)	470 (183-2329)
Histology, n	
Low-grade	10
Follicular lymphoma	9
Small lymphocytic lymphoma	1
Intermediate-grade	42
Peripheral T-cell lymphoma, unspecified	14
NK-cell lymphoma	12
Anaplastic large cell lymphoma	6
Diffuse large B-cell lymphoma	5
Angioimmunoblastic lymphoma	1
Mantle cell lymphoma	1
High-grade	60
Lymphoblastic lymphoma	39
Adult T-cell leukemia/lymphoma	15
Burkitt lymphoma	5
Unclassified	12
Previous history of HDT/ASCT, n	
Yes	18
No	83
ND	23
Disease status at transplantation, n	
CR	60 (CR1 19, CR2 18, CR3 5, CR4 1)
Non-CR	60
ND	4
Chemosensitivity at transplantation, n	
Sensitive	76
Resistant	33
ND	15
Conditioning regimen, n	
TBI-containing regimen	111
Non-TBI regimen	13
GVHD prophylaxis, n	
CsA ± MTX ± steroid	76
TCR ± MTX ± steroid	44
CsA + TCR ± MTX ± steroid	3
MTX alone	1

HDT/ASCT indicates high-dose therapy and autologous stem cell transplantation; CR, complete remission (CR1, CR2, CR3, CR4; the first, second, third, and fourth CR, respectively); ND, not described; TBI, total body irradiation; GVHD, graft-versus-host disease; CsA, cyclosporine A; MTX, methotrexate; TCR, tacrolimus.

(LSG),[8] Revised European-American Classification of lymphoid neoplasms (REAL),[9] and World Health Organization (WHO) systems.[10] In this study, we grouped the histology into low grade, intermediate grade, and high grade as usually accepted in daily practice. There were 10, 42, and 60 patients with low-, intermediate-, and high-grade lymphoma, respectively. Histologic subtypes in detail are described in Table 1. The histologic subtype or grade was unclassified in 12 cases. Transplantation for lymphoblastic lymphoma (LBL) and adult T-cell leukemia/lymphoma (ATLL) was included as in other studies focusing on allogeneic transplantation for NHL.[11,12]

Data management and statistical considerations

Data were collected by the JMDP using a standardized report form. Follow-up reports were submitted at 100 days, 1 year, and annually after transplantation. Overall survival (OS) was defined as days from transplantation to death from any cause. Progression-free survival (PFS) was defined as days from transplantation to disease progression or death from any cause. Nonprogression mortality was defined as death without disease progression. Patients who were alive at the last follow-up date were censored. Survival was calculated using the Kaplan-Meier method. To evaluate the influence of confounding factors for survival, the log-rank test was used for univariate analyses and proportional hazard modeling was used for multivariate analyses. Cumulative incidences of acute GVHD and disease progression were calculated using the Gray method,[13] considering death without acute GVHD and death without disease progression as respective competing risks. The effects of acute and chronic GVHD on survival and disease progression were analyzed among patients who survived without disease progression at 60 and 150 days after transplantation, respectively.[14,15] This landmark method was used to exclude bias that may arise from including patients who died too early to develop GVHD in the group without GVHD.

Results

Survival and disease progression

Of the 124 patients, 69 were alive with a median follow-up duration of 565 days (range, 82 to 2217 days) after transplantation (Table 2). The overall 3-year OS and PFS were 49.7% and 42.6%, respectively (Figure 1A). Cumulative incidences of disease progression and nonprogression mortality at 3 years were 24.5% and 32.9%, respectively (Figure 1B). Disease progression was observed in 26 patients, and the median time from transplantation to disease progression was 109 days (range, 0 to 1079 days). Notably, only 1 patient developed disease progression more than 500 days after

Table 2. Transplantation outcome

	Value
Alive/dead, n	69/55
Median follow-up for survivors, d (range)	565 (82-2217)
Cause of death	
Progression, n	17
Median days after transplantation (range)	165 (2-1106)
Death without progression, n	36
Median days after transplantation (range)	72 (8-718)
GVHD, n	10
Infection, n	9
IP, n	6
VOD, n	3
Renal failure, n	2
ARDS, n	2
Others: pericarditis, hemorrhage, cerebral infarction, RRT, n	4
Not described, n	2
Disease progression, n	26
Median days after transplantation (range)	109 (0-1079)
Engraftment, n	
Engraftment	115
Rejection	2
Death within 20 days	7
Acute GVHD, n*	
Grade 0	31
Grade I	37
Grade II	30
Grade III	7
Grade IV	10
Chronic GVHD, n†	
None	47
Limited	17
Extensive	24
Not described	5

GVHD indicates graft-versus-host disease; IP, interstitial pneumonitis; VOD, venoocclusive disease; ARDS, acute respiratory distress syndrome; RRT, regimen-related toxicity.

*Acute GVHD was evaluated among patients who achieved engraftment and survived more than 20 days after transplantation.

†Chronic GVHD was evaluated among patients who survived more than 100 days after transplantation.

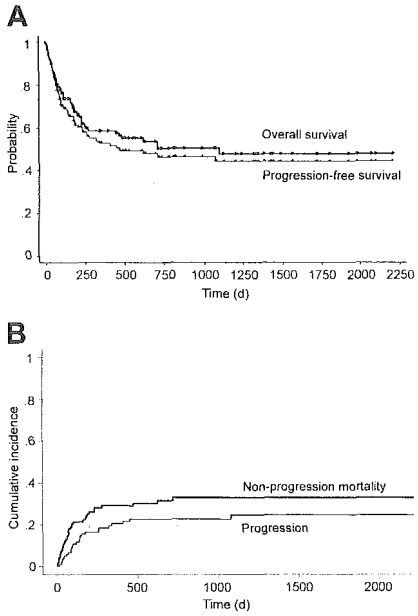

Figure 1. Survival, progression, and nonprogression mortality after transplantation. Overall survival, progression-free survival (A), and cumulative incidences of disease progression and nonprogression mortality (B) after unrelated bone marrow transplantation for non-Hodgkin lymphoma.

transplantation. The cause of death was related to disease progression in 17, whereas 36 died without disease progression (Table 2). The major cause of transplantation-related death within 100 days after transplantation was GVHD in 8, infection in 5, venoocclusive disease in 3, acute respiratory distress syndrome in 2, interstitial pneumonitis in 2, renal failure in 2, and other causes in 2.

Engraftment and GVHD

Seven patients died within 20 days after transplantation, and therefore engraftment could not be evaluated. In the others, 2 rejected the graft and 115 achieved engraftment. Among the latter 115 patients, 47 developed grades II-IV acute GVHD (Table 2) with a cumulative incidence of 47.5% (Figure 2). Seven and 10 patients experienced grade III and IV acute GVHD, respectively. Among the 93 patients who were alive at 100 days after transplantation, 17 and 24 developed limited and extensive chronic GVHD, respectively.

Influence of pretransplantation factors

We evaluated the effects of pretransplantation factors on OS after transplantation and identified 3 independent significant risk factors: chemosensitivity before transplantation (chemosensitive versus chemoresistant, relative risk 0.28, 95% confidence interval [CI] 0.15-0.52, $P < .0001$); previous history of HDT/ASCT (yes versus no, relative risk 0.40, 95% CI 0.20-0.79, $P = .0087$); and patient age (less than 40 years versus 40 years or more, relative risk 0.42, 95% CI 0.22-0.81, $P = .0092$) at transplantation (Tables 3 and 4; Figure 3A-C). These 3 factors were also identified as independent risk factors for PFS (data not shown), probably because only a few patients survived after disease progression and the OS and PFS curves were almost superimposed. We further analyzed the impact of disease status among patients who had chemosensitive disease at bone marrow transplantation; 19 were in first CR, 24 were in a later CR, and 16 were in PR. However, there was no significant difference in OS or PFS among them (data not shown). Among 13 deaths in patients with previous history of HDT/ASCT, 11 were from transplantation-related causes before day 100 (median, 56; range, 13 to 97 days after transplantation). Two patients died from

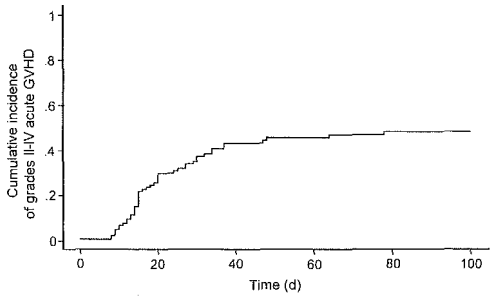

Figure 2. Cumulative incidence of grades II-IV acute graft-versus-host disease.

disease progression on day 48 and 458, respectively. However, 5 of 6 patients who survived more than 100 days after transplantation were progression free at a median follow-up of 1339 days (range, 493 to 2217 days) after transplantation. Furthermore, we evaluated the impact of histologic grade on outcome. However, there was no significant difference in OS, PFS, or cumulative incidence of disease progression among these histologic grades (Figure 3D and data not shown).

Influence of acute and chronic GVHD

We analyzed the relationship between the development of acute GVHD and the transplantation outcome. In this study, all but 2 patients developed acute GVHD before day 60. Thus, we defined day 60 as a landmark for this analysis. The cumulative incidence of disease progression at 3 years after transplantation was significantly lower in patients who developed grades II-IV acute GVHD (5.9% versus 33.2%, $P = .0053$; Figure 4B). This effect was preserved even when it was adjusted for the chemosensitivity before transplantation using proportional hazard modeling (relative risk 0.15, 95% CI 0.03-0.65, $P = .012$). This inverse correlation between the development of acute GVHD and disease progression

Table 3. Prognostic factors in univariate analyses

	3-year OS, %	P
Sex		.70
Male	47.7	
Female	53.1	
Age at transplantation		.0036
Less than 40 y	57.1	
40 y or more	28.7	
Histology		.80
Low grade	60.0	
Intermediate grade	53.2	
High grade	47.6	
Previous HDT/ASCT		.0006
Yes	27.8	
No	56.3	
Chemosensitivity		< .0001
Chemosensitive	63.2	
Chemoresistant	22.8	
Disease status		.0011
CR	61.4	
Non-CR	32.0	
Preparative regimen		.29
TBI-containing	50.8	
Non-TBI	40.0	
Days from diagnosis to transplantation		.53
Less than 365 d	44.8	
365 d or more	52.1	

OS indicates overall survival; HDT/ASCT, high-dose therapy and autologous stem cell transplantation; CR, complete remission; TBI, total body irradiation.

Table 4. Prognostic factors in multivariate analysis

	Relative risk	95% CI	P
Age less than 40 y	0.42	0.22-0.81	.0092
No previous HDT/ASCT	0.40	0.20-0.79	.0087
Chemosensitive disease	0.28	0.15-0.52	< .0001

CI indicates confidence interval.

suggested the existence of a graft-versus-lymphoma (GVL) effect. However, there was no significant difference in 3-year OS (61.4% versus 58.8%, $P = .63$; Figure 4A) or PFS (58.9% versus 48.9%, $P = .28$) between patients with and without grades II-IV acute GVHD. When we classified patients into those who developed grades III-IV acute GVHD and those who did not, there was a trend for lower incidence of disease progression ($P = .13$) but worse OS ($P = .075$) in patients with acute GVHD. The influence of chronic GVHD was evaluated similarly, with day 150 after transplantation defined as a landmark. However, the cumulative incidence of disease progression at 3 years after transplantation was not different between those with and without chronic GVHD (12.3% versus 14.5%, $P = .80$).

Results in specific histologic subtypes

Kaplan-Meier estimates of OS of patients with peripheral T-cell lymphoma (n = 14), natural killer (NK)–cell lymphoma (n = 12), LBL (n = 39), and ATLL (n = 15), which were the 4 major histologic subtypes in this study, are shown in Figure 5. OS of patients with peripheral T-cell lymphoma, which is considered to be associated with poor prognosis,[16-18] appeared to be favorable after unrelated allogeneic transplantation with several long-term survivors (3-year OS, 75.0%), although this study contained only a small number of patients. In contrast, the result for ATLL was poor, and there were no survivors beyond 500 days after transplantation.

Discussion

In this study, we analyzed the outcome of bone marrow transplantation from an unrelated donor for NHL performed through the JMDP. In a similar study from the National Marrow Donor Program (NMDP),[19] both OS and PFS were estimated to be 30% at 2 years. The outcome in the present study appeared to be more favorable than that in the NMDP study, which could be attributed to the lower incidence of grades III-IV acute GVHD in the present study (15% versus 30%). This observation is compatible with previous studies showing lower incidence of acute GVHD among Japanese than among whites, which might reflect less diverse genetic background in Japan.[20,21]

The association between the development of GVHD and reduced disease progression rate has been inconsistent among previous studies.[4,11,22] In this study, the development of grades II-IV acute GVHD was associated with a lower incidence of disease progression after transplantation. This result supports the existence of a potential GVL effect. Because the previous studies nearly exclusively included transplantation from an

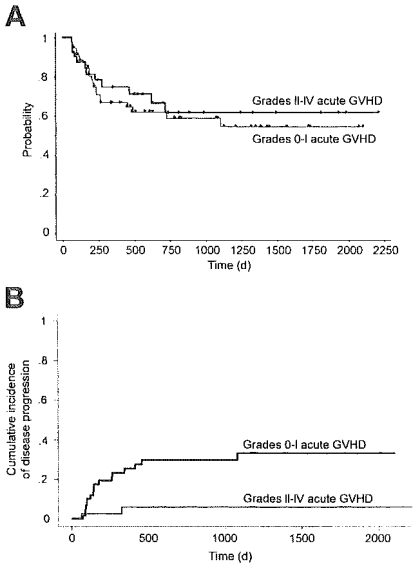

Figure 4. Survival and progression according to development of acute GVHD. OS (A) and cumulative incidence of disease progression (B) grouped by the development of grades II-IV acute graft-versus-host disease among patients who were alive without disease progression at 60 days after transplantation.

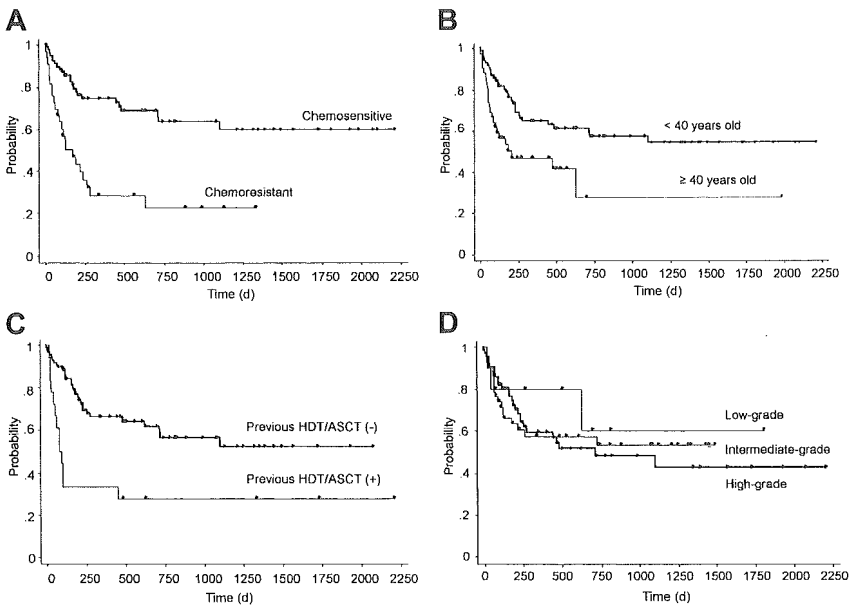

Figure 3. Overall survival according to pretransplantation factors. OS grouped by chemosensitivity at transplantation (A), age (B), previous history of autologous transplantation (HDT/ASCT) (C), and histologic grade (D).

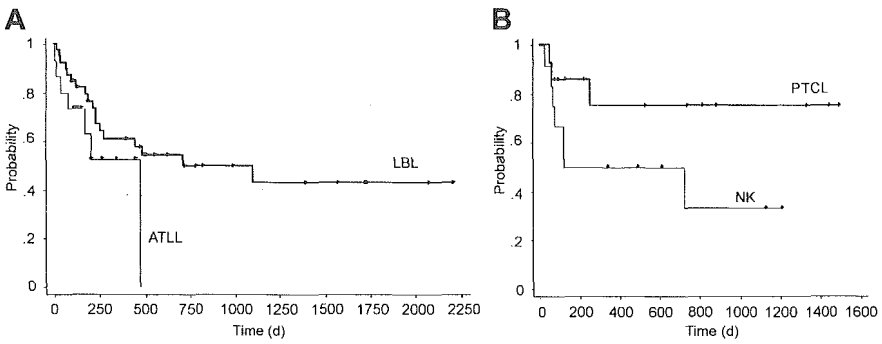

Figure 5. Overall survival of specific histologic subtypes. (A) Adult T-cell leukemia/lymphoma (ATLL) and lymphoblastic lymphoma (LBL); (B) peripheral T-cell lymphoma (PTCL) and NK-cell lymphoma (NK).

HLA-matched sibling, the use of unrelated donor might have facilitated the GVL effect. Nevertheless, there was no difference in OS or PFS between patients with and without grades II-IV acute GVHD, because the decreased incidence of disease progression was counterbalanced by the increased incidence of transplantation-related mortality. An association between the development of chronic GVHD and the incidence of disease progression was not observed. We suppose that the main reason we failed to observe this association is insufficient statistical power due to the paucity of disease progression (only 9 patients) beyond day 150, a landmark for the analysis.

Chemosensitivity at transplantation was identified as a major prognostic factor for OS. Patients with chemosensitive disease at transplantation were associated with lower nonprogression mortality, a lower incidence of disease progression, and better survival. These results may raise the question whether the effect of allogeneic transplantation was from the GVL effect or from high-dose therapy before transplantation. However, this study strongly suggested the existence of the GVL effect, because the incidence of disease progression was significantly lower in patients who developed acute GVHD, even after adjusted for chemosensitivity before transplantation. Previous history of HDT/ASCT was also a strong prognostic factor. Because the median survival for patients with NHL who had a relapse after HDT/ASCT is extremely short (less than 12 months),[23] allogeneic transplantation using conventional or reduced-intensity conditioning is being evaluated in this population. In this study, 11 patients (61%) with a previous history of HDT/ASCT died within 100 days after transplantation from transplantation-related causes. On the other hand, most of the patients who survived beyond day 100 were progression free with long follow-up, suggesting a benefit of allogeneic transplantation to suppress disease progression. Therefore, strategies to decrease transplantation-related mortality are important, especially for patients after HDT/ASCT failure. Allogeneic transplantation with reduced-intensity conditioning is an option that deserves further evaluation.[24]

LBL, ATLL, peripheral T-cell lymphoma, and NK-cell lymphoma were the 4 major histologic subtypes in this population. The composition of the histologic subtypes in this study was different from that of NHL in general and that in previous studies focusing on allogeneic transplantation for NHL.[4,12] Higher ratios of peripheral T-cell lymphoma, NK-cell lymphoma, and ATLL would, at least in part, be a reflection of the histologic population of NHL in Japan.[25] Because the long-term results with conventional therapy and/or HDT/ASCT for ATLL had been always dismal,[26] allogeneic transplantation even for patients in CR is being tested in a clinical trial in several centers in Japan.[27] The long-term results for peripheral T-cell lymphoma and NK-cell lymphoma with conventional therapy, especially in patients with advanced or relapsed disease, were also poor.[16-18,28] The high ratio of LBL might be a reflection of Japanese physicians' preference to perform allogeneic transplantation for LBL in CR1.

Transplantation outcome in each histologic subtype should be evaluated further to select patients who will benefit from unrelated transplantation. In this study, there was no difference in OS or PFS among the 3 grades. Although no patient with low-grade lymphoma had disease progression at a median follow-up of 513 days, the number of patients was too small and follow-up period was too short to draw a definite conclusion. Based on the results of this study, unrelated donor bone marrow transplantation deserves to be evaluated in patients with peripheral T-cell lymphoma and NK-cell lymphoma, considering the poor results after conventional chemotherapy for these subtypes.[16-18,28] Finally, although the outcome of patients with ATLL was poor in this study, this treatment strategy should not be abandoned because both the number of the patients and the follow-up duration were not enough.

In conclusion, allogeneic bone marrow transplantation from an unrelated donor appeared to be a feasible treatment option for patients with high-risk NHL. Further study is required to determine detailed indications for unrelated transplantation for NHL, including histologic subtype and disease status.

Appendix

The following centers in Japan participated in the bone marrow transplantations for NHL facilitated by the JMDP: Hokkaido University Hospital, Sapporo Hokuyu Hospital, Japanese Red Cross Asahikawa Hospital, Iwate Medical University Hospital, Tohoku University Hospital, Yamagata University Hospital, National Cancer Center Central Hospital, Tokyo Metropolitan Komagome Hospital, Nihon University Itabashi Hospital, Jikei University Hospital, Keio University Hospital, University of Tokyo Hospital, National Tokyo Medical Center, Kanagawa Children's Medical Center, Kanagawa Cancer Center, Tokai University Hospital, Chiba University Hospital, Saitama Cancer Center Hospital, Saitama Medical School Hospital, Jichi Medical School Hospital, Saiseikai Maebashi Hospital, Gunma University Hospital, Niigata Cancer Center Hospital, Saku Central Hospital, Japanese Red Cross Nagoya First Hospital, Nagoya Daini Red Cross Hospital, Meitetsu Hospital, Nagoya University Hospital, Aichi Cancer Center, Showa Hospital, Kanazawa University Hospital, Kinki University Hospital, Osaka University Hospital, Osaka Medical Center and Research Institute for Maternal and Child Health, Matsushita Memorial Hospital, Kansai Medical University Hospital, Hyogo College of Medicine Hospital, Hyogo Medical Center for Adults, Kyoto University Hospital, Tottori University Hospital, Hiroshima Red Cross Hospital and Atomic-Bomb Survivors Hospital, Ehime Prefectural Central Hospital, National Okayama Medical Center, Kyushu University Hospital, Harasanshin General Hospital, Hamanomachi General Hospital, National Kyushu Cancer Center, St Mary's Hospital, Saga Prefectural Hospital, Nagasaki University Hospital, Kumamoto National Hospital, and Oita Medical University Hospital.

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Post-transplant complications

Intestinal thrombotic microangiopathy after allogeneic bone marrow transplantation: a clinical imitator of acute enteric graft-versus-host disease

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Summary:

Thrombotic microangiopathy after bone marrow transplantation (post-BMT TMA) is a serious transplant-related complication. We identified 16 patients with TMA after allogeneic BMT who showed histopathological evidence of intestinal TMA in their gut specimens (six autopsies, 10 biopsies). In all, 14 patients had grade II–IV acute graft-versus-host disease (GVHD). The first seven patients were retrospectively diagnosed with TMA. Since six of them were diagnosed with progressive GVHD at that time because there was no awareness of the existence of intestinal TMA, they received more intensive treatment for GVHD, but all died between days +49 and +253. In contrast, the remaining nine patients were recently diagnosed with intestinal TMA on the basis of colonoscopic biopsies. For eight of these patients, the immunosuppressants were reduced, and the patients' intestinal symptoms improved gradually. Six of the nine patients were still alive 12 months after the diagnosis of TMA. Our findings suggest that the gut may be a site involved in post-BMT TMA, presenting as ischemic enterocolitis. Differentiating intestinal TMA from acute GVHD is important in patients suffering from severe and refractory diarrhea after BMT.

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Thrombotic microangiopathy (TMA) is a well-recognized disorder that occurs after either allogeneic or autologous bone marrow transplantation (BMT).[1–4] Although several factors may contribute to the onset of this complication, acute graft-versus-host disease (GVHD), cyclosporin A (CsA), tacrolimus (FK506), total body irradiation (TBI), high-dose chemotherapy and infection play a critical role in its etiology.[5–16] As TMA is associated with high mortality, its early diagnosis is particularly important. A grading system (grades 0–4) of TMA based on two clinical parameters, lactic dehydrogenase (LDH) level and the percentage of fragmented erythrocytes,[2] has been proposed for this purpose. However, it is often difficult to diagnose TMA in patients who have undergone BMT due to the heterogeneity of TMA.

In previous studies on post-BMT TMA, much attention has been paid to renal impairment and central nervous system toxicity, but far less to the potential involvement of the target organs for acute GVHD. It is well known, however, that gastroenteritis accompanied by bloody diarrhea and abdominal pain often heralds the onset of renal failure in young children with classical hemolytic uremic syndrome (HUS). Recent pathological studies have shown the presence of microangiopathy in the intestinal mucosa of patients with HUS associated with *Escherichia coli* O157:H7 infections.[17,18] Moreover, thrombotic thrombocytopenic purpura (TTP) not related to BMT is characterized by disseminated platelet thrombosis in the microvasculature of multiple organs including the intestinal tract, liver and skin.[19] Therefore, it seems reasonable to assume that the gut may be involved in microangiopathy in patients with the florid form of post-BMT TMA.

For the study presented here, we identified 16 cases with histopathological evidence of post-BMT TMA. This report is the first to show that the intestine can be involved in TMA following BMT.

Materials and methods

Patients

Between 1987 and 1999, 251 patients underwent allogeneic BMT at the Department of Internal Medicine, Japanese

Red Cross Nagoya First Hospital. Of these patients, 16 showed pathological evidence of intestinal TMA (six by autopsy, 10 by biopsy). The 16 patients' records were retrospectively examined. All patients gave written informed consent for participation in these treatments.

BMT procedures

Several preparative regimens were employed for the allogeneic BMT patients (Table 1). Oral busulfan (BU) 4 mg/kg/day was administered for 2 days (days −7, −6) followed by intravenous (i.v.) cyclophosphamide (CY) 60 mg/kg by 3-h i.v. infusion for 2 days (days −5, −4) and by 5 Gy of TBI given daily for 2 days (days −2, −1). Cytosine arabinoside (CA) 2 g/m^2 was administered by 3-h i.v. infusion for 3 days (days −6 (× 2), −5, −4) followed by CY 60 mg/kg for 2 days (days −5, −4) and by TBI 6 Gy daily for 2 days (days −2, −1). Melphalan (LPAM) 60 mg/m^2 was administered by a one-shot i.v. infusion for 3 days (days −6, −5, −4) followed by TBI 5 Gy daily for 2 days (days −2, −1). Patients 3 and 5 received chemotherapy, comprising carboplatin and CA or mitoxantron, etoposide and CA, to reduce their tumor burden 14 days before the preconditioning.

GVHD prophylaxis for seven patients consisted of a combination of i.v. methotrexate (MTX) at 10 mg/m^2 on day 1 and at 7 mg/m^2 on days 3 and 5 and i.v. CsA at 3 mg/kg/day beginning on day −1. GVHD prophylaxis of the remaining nine patients comprised a combination of MTX and continuous i.v. tacrolimus (FK506) at 0.03 mg/kg/day beginning on day −1.

Corticosteroids were administered once acute GVHD (more than grade II) had been diagnosed according to the following schedule: methylprednisolone (mPSL) at 20 mg/kg i.v. for 3 days, at 15 mg/kg for 3 days and then tapered off. mPSL 2–4 mg/kg i.v. for 7–14 days was administered simultaneously with FK506 depending on disease severity when acute GVHD (≥grade II) had been identified in the more recent six patients.

Colonoscopic examination

Of 122 patients who received BMT after 1996, 10 underwent colonoscopic examination together with mucosal biopsy to determine the etiology of severe and refractory bloody diarrhea. An intermediate length scope (CF200I; Olympus, Tokyo, Japan) was used for this examination.

Histopathological and immunohistological study

Biopsy specimens were obtained from 10 patients who were suffering from at least 500 ml/day of bloody diarrhea after allogeneic BMT. Five or six specimens including those of the cecum were obtained from nine of these patients, and three specimens from the rectum of the remaining patient who underwent retrosigmoidoscopy only. The autopsy specimens obtained from six patients who had developed bloody diarrhea (500 ml/day) after allogeneic BMT were also analyzed. All tissues were fixed in 10% formalin, embedded in paraffin and stained with hematoxylin–eosin (HE) and elastica van Gieson. Serial sections were used for biopsy materials. For autopsy materials, seven or more

Table 1 Clinical characteristics of BMT-TMA patients

Patients no.	*Age (years)*	*Sex*	*Disease*	*Stage*	*Donor*	*Blood type pt./donor*	*HLA*	*Preconditioning regimen*	*GVHD prophylaxis*
1	21	M	AML	Second CR	R (mother)	A+/O+	Phenotypically identical	CA+CY+TBI	CsA+sMTX
2	20	F	CML	BC	UR	AB+/O+	A2 DNA, B serological mismatched	BU+CY+TBI	CsA+sMTX
3	36	F	AML	Non-CR	UR	A+/B+	Matched	CBDCA+CA, BU+CY+TBI	CsA+sMTX
4	43	M	CML	CP	UR	B+/B+	Matched	CA+CY+TBI	CsA+sMTX
5	47	F	AML	Non-CR	UR	AB+/A+	Matched	Mit+VP16+CA, BU+CY+TBI	CsA+sMTX
6	41	M	NHL	Stage IV	UR	B+/A+	A2, A26, B61 DNA mismatched	LPAM+TBI	CsA+sMTX
7	22	M	CML	CP	UR	O+/AB+	A2, A26 DNA mismatched	CA+CY+TBI	CsA+sMTX
8	49	M	ALL	First CR (Ph1)	R (sibling)	A+/O+	Phenotypically identical (MLC positive)	LPAM+BU+TBI, CSI, TI	FK506+sMTX
9	36	M	CML	BC	UR	A+/B+	DRB1 DNA 2 loci mismatched	BU+CY+TBI	FK506+sMTX
10	42	M	CML	CP	UR	AB+/AB+	DRB1 DNA 1 locus mismatched	CA+CY+TBI	FK506+sMTX
11	32	M	CML	CP	UR	A+/A+	DRB1 DNA 1 locus mismatched	CA+CY+TBI	FK506+sMTX
12	43	M	ATL		UR	A+/A+	Matched	LPAM+TBI	FK506+sMTX
13	44	M	CML	AP	UR	A+/O+	A26 DNA mismatched	BU+CY+TBI	FK506+sMTX
14	33	M	ALL	First CR	UR	A+/O+	DRB1 DNA 1 locus mismatched	L-PAM+TBI, CSI	FK506+sMTX
15	49	M	MDS	Overt leukemia	UR	B+/A+	DR serological mismatched	BU+CY+TBI	FK506+sMTX
16	28	M	CML	AP	UR	O+/A+	DR serological mismatched	BU+CY+TBI	FK506+sMTX

BMT-TMA = bone marrow transplant-associated thrombotic microangiopathy; AML = acute myelocytic leukemia; CML = chronic myelocytic leukemia; NHL = non-Hodgkin's lymphoma; ALL = acute lymphoblastic leukemia; ATL = adult T-cell leukemia MDS = myelodysplastic syndrome; CR = complete remission; CP = chronic phase; BC = blast crisis; AP = accrelated phase; R = related donor; UR = unrelated donor; HLA = human lymphocyte antigen; GVHD = graft-versus-host disease; MLC = mixed lymphocyte culture CA = cytarabine arabinoside; CY = cyclophosphamide; TBI = total body irradiation; BU = busulfan; LPAM = melphalan; CBDCA = carboplatin; Mit = mitoxantron; VP16 = etoposide; CSI = cerebrospinal irradiation; TI = testicular irradiation; CsA = cyclosporin A; sMTX = short-term methotrexate; FK506 = tacrolimus.

tissue samples were taken from the affected parts of the intestine and mounted on cardboard to avoid flection. A minimum of three blocks was cut from each sample. Immunostaining of representative sections used monoclonal antibodies for GPIIbIIIa (CD41a), von Willebrand factor (vWF), CD34, α-smooth muscle actin (α-SMA) and cytomegalovirus (CMV). Pathological specimens were reviewed in a blinded manner.

Diagnosis of TMA

Diagnosis of TMA was histologically based on either hyaline (platelet) thrombi in the capillaries of the biopsy specimens or thrombonecrotic arteriolar lesions (thrombonecrotic arteriolopathy) of the intestine in the autopsy specimens. TMA was also clinically diagnosed according to the grading system for BMT-TMA, which is based on the LDH level and the proportion of fragmented erythrocytes as described previously.[2] A single observer counted 500 red blood cells on blinded smears and calculated the percentage of fragmented erythrocytes retrospectively. A fragmented erythrocyte was defined as a schistocyte (crescent, helmet or triangle)[2,20] and the LDH/platelet ratio as described previously.[2]

Diagnosis of GVHD

Acute GVHD was diagnosed and graded from I to IV on the basis of the consensus criteria[21] for each of the clinical sites. The clinical and laboratory parameters used to assess the grade of acute GVHD included the percentage of body-surface area with skin rash, the volume of diarrhea, total bilirubin and Karnofsky's performance status. Skin biopsy samples were obtained to confirm the diagnosis of acute GVHD before the start of treatment for 14 patients. Gut biopsies were obtained from nine patients during the treatment for acute GVHD. Patients surviving beyond day 100 were considered to qualify as chronic GVHD, which was categorized as limited type (localized skin and/or hepatic involvement) or extensive type (diffuse skin and/or multiorgan involvement).[22]

Results

Patient characteristics

The 16 patients with pathological evidence of intestinal TMA consisted of three females and 13 males with a median age of 39 (range: 20–49) years. Pre-transplantation, all patients showed normal organ functions. Patient background characteristics are listed in Table 1. Four of the 16 patients had undergone ABO compatible transplantation. However, four patients (3, 6, 9 and 15) underwent major–minor, two (7 and 16) major and six (1, 2, 5, 8, 13 and 14) minor ABO-mismatched transplantations, respectively. Two patients (1 and 8) underwent transplantation from a phenotypically identical related donor and four (3, 4, 5 and 12) from a HLA-A-, -B-, DRB1-matched unrelated donor. The remaining 10 patients received class I or II DNA-mismatched or serologically mismatched unrelated donor transplants. Their diagnoses consisted of chronic myelogenous leukemia (eight), acute myelocytic leukemia (three), acute lymphoblastic leukemia (two), and one each for adult T-cell leukemia, non-Hodgkin's lymphoma and myelodysplastic syndrome.

Clinical and laboratory findings of TMA patients

The clinical findings and blood examinations for the TMA patients are shown in Table 2. Clinically, 14 of the 16 patients were diagnosed with multifactorial TMA. TMA onset ranged from days +16 to +69 after BMT. Four patients had grade 4, six had grade 3 and four had grade 2 BMT-TMA. The median peak of the percentage of fragmented erythrocytes was 6.7% with a range of 0.8–13.3%. The median peak LDH level was 879 IU/l (range, 382–2274 IU/l), which was twice as high as the upper range of normal laboratory findings. All patients clinically diagnosed with TMA also showed other findings of microangiopathic hemolytic anemia: reticulocytosis, refractory thrombocytopenia or a low haptoglobin level.

Stool specimens were routinely obtained from all patients and cultured for the presence of bacterial and fungal

Table 2 Clinical findings in BMT-TMA patients

Patients no.	*TMA onset day*	*TMA grade*	*% fragmented erythrocytes*	*LDH/platelet ratio*	*LDH (IU/l)*	*T.Bil (mg/dl)*	*Ret (‰)*	*Hpt (mg/dl)*	*Cr (mg/dl)*
1	+63	3	6.5	30	829	17.6	82	23	1.3
2	+41	3	6.8	42	836	20.3	44	10>	0.5
3	+19	3	6.5	66	1779	2.0	2	11	0.6
4	+50	4	11.4	95	1902	29.7	114	10>	0.7
5	+69	4	13.3	53	2274	2.5	57	10>	1.6
6	+16	4	10.5	66	712	2.5	37	11	0.9
7	+44	3	8.0	63	1520	1.3	33	10>	0.6
8	+25	4	10.5	52	1252	19.0	21	12	2.2
9	+40	2	4.5	13	554	2.2	46	12	1.2
10	+53	3	7.6	79	952	3.7	69	10>	0.7
11	+24	2	3.5	11	551	0.9	13	10>	0.7
12	+28	3	7.6	12	668	0.7	34	31	1.1
13	+28	2	2.0	20	637	0.7	22	10>	1.0
14	+34	2	2.4	91	1821	0.4	55	12	4.6
15	—	0	1.2	50	922	25.5	16	42	0.6
16	—	0	0.8	14	382	2.3	23	117	0.9

Normal range : peak % fragmented erythrocytes = < 1.2%; peak LDH = < 500 IU/l; Hpt = haptoglobin.

pathogen. *Staphylococcus epidermidis* was detected in the stool obtained from 13 patients, and Bacteroides was detected from two patients. These pathogens were also detected after the intestinal symptoms improved due to the reduction of immunosuppressants, as well as during diarrhea. *S. aureus*, which was sensitive to antibiotics, was detected once in three examinations during diarrhea from patient 12. *Candida glabrata* was also detected once in six examinations during diarrhea from patient 16. The pathogens that cause enterocolitis, such as *Clostridium difficile*, *Salmonella*, *Shigella*, *Campylobacter*, *E. coli* O157:H7, were not detected. Isolation of viral pathogens was performed for stool specimens obtained from 11 patients during diarrhea, but no viral pathogens were isolated. These results suggest that the pathogens detected in this study did not cause diarrhea directly.

Clinical symptoms and outcome

All 16 patients developed severe bloody diarrhea (910–3400 ml/day). Of the 15 patients who could be evaluated, 14 (93%) had grade II–IV acute GVHD. They were treated with mPSL 1 g pulse therapy or mPSL 2–4 mg/kg i.v. against the advancement of acute GVHD. Antithymocyte globulin was also given to five patients as tertiary treatment.

The initial seven patients were diagnosed post mortem with TMA. Six of these patients (1, 2, 4, 5, 6 and 7) had severe bloody diarrhea, which was thought to be caused by the recurrence of acute enteric GVHD at that time. They therefore received more intensive immunosuppressive therapy, but all died between days +49 and +253. Two died from veno-occlusive disease (VOD), one each from renal failure due to nephritis, multiple organ failure (MOF), drug-induced encephalopathy and suicide. Three patients (2, 4 and 5) underwent therapeutic apheresis with plasma exchange, but none of them responded. Another patient, who was diagnosed with TMA by autopsy, suffered from cerebral bleeding and died on day +24.

The next nine patients (8–16) were diagnosed as TMA from the results of gut biopsy. Bloody diarrhea and abdominal pain continued in patients 9–16 even after improvement of acute GVHD of skin. Immunosuppressants were reduced and the patients' intestinal symptoms improved gradually. Antibiotics were administered to four patients (8, 9, 10 and 16) who had the episodes of fever during diarrhea, but there was no relation between their courses of fever and diarrhea. In patient 8, immunosuppressive agents could not be reduced because of the severity of GVHD grade IV, and he died of VOD and liver failure on day +83. Six of these nine patients (67%) were still alive 12 months after the diagnosis of TMA. Three patients (10, 15 and 16) are alive and well 68, 44 and 42 months post transplantation. Table 3 summarizes the outcome.

Six (75%) of eight patients who could be evaluated developed chronic GVHD (five extensive type and one limited type). One patient (12) suffered from bloody diarrhea again after chronic GVHD improved with the administration of corticosteroids, and he died on day +473. In two patients (9 and 11), chronic GVHD progressed in spite of immunosuppressive therapy and they died of chronic GVHD and respiratory failure on days +1020 and +1098.

Patient 13 and 14 died of liver failure and MOF, respectively, even though their diarrhea due to intestinal TMA improved with the tapering of the immunosuppressive agents.

Colonoscopic findings

Colonoscopic examination was performed for 10 of the recent patients (7–16). Their mucosa was edematous and erosive, and they showed multiple irregular erythemas with spontaneous bleeding in the rectum. Similar lesions were observed in the sigmoid and transverse colon. Interestingly, these findings were more severe in the cecum.

Histopathological and immunohistological findings

Microangiopathy was identified in all 16 patients. Postmortem angiopathy was predominantly found in the submucosal arterioles as either thrombonecrosis or mucoid subintimal thickening. However, the small intestine of patient 3, who had died on day +24 post-BMT, showed only mucosal changes. There were fibrinoid necrosis of the terminal arterioles located in deep mucosa, extravasation with red cell fragmentation around the affected vessels, extensive crypt depletion and hemorrhage in the tip of mucosal folds.

As for the biopsy specimens, the vascular changes in the mucosa were subtle. Various degrees of perivascular hemorrhages with or without hemosiderin were commonly observed (Figure 1a). Careful examination of serial sections turned up a few capillary thrombosis either granular or hyaline in appearance (Figure 1b). The thrombi were confirmed as platelet thrombi by immunostaining with CD41a (Figure 1c) and vWF. Immunohistochemistry with CD34 and α-SMA showed a decrease in the staining intensity of the affected vessels (data not shown). Diagnostic changes in patient 7, 8, 10 and 11 were present only in the cecal mucosa. These histologic features were frequent in the specimens taken from erosive mucosa composed of crypt depletion and hemorrhage. A few CMV inclusion cells were found in the specimens of three patients (8, 9 and 13). The findings of enterocolitis due to bacterial and fungal infection were not found. Although exploding crypt lesions suggestive of GVHD were seen in several of the regenerating mucosa of the other two patients (11 and 12), it was difficult to distinguish them from those of rapid crypt regeneration because of the absence of significant lymphocytic infiltration.

Discussion

We identified 16 patients with pathological evidence of intestinal TMA. It is worth noting that 14 of these patients showed multifactorial post-BMT TMA (grades II–IV) and most of them experienced acute GVHD before the onset of TMA. Endoscopic features of the intestine included edematous mucosa, erythema, multiple ulcers and spontaneous bleeding, which was similar to the findings for

Table 3 Clinical symptoms and outcome in BMT-TMA patients

Patients no.	*aGVHD grade*	*Stage of gut*	*Therapy for aGVHD*	*Max volume (ml/day) of diarrhea*	*Duration (days) of diarrhea ⩾500 ml*	*Bloody diarrhea*	*Following therapy*	*Chronic GVHD*	*Other complications*	*Outcome*
1	III	3	mPSL pulse with CsA, ATG	2050	19	+		NE	VOD	Dead, suicide (day +69)
2	IV	3	mPSL pulse with CsA	2100	31	+	Plasma exchange	NE	VOD	Dead, VOD and liver failure (day +60)
3	UE	—	—	950	9	+		NE	Cerebral bleeding	Dead, cerebral bleeding (day +24)
4	III	3	mPSL pulse with CsA, FK506	1760	28	+	Plasma exchange	NE	Encephalopathy, VOD	Dead, VOD and liver failure (day +73)
5	IV	3	mPSL pulse with CsA, ATG	1840	31	+	Plasma exchange	NE	Encephalopathy, hemorrhagic cystitis	Dead, nephritis and renal failure (day +89)
6	IV	4	mPSL pulse, ATG	3400	35	+		NE	Encephalopathy	Dead, multiple organ failure (day +49)
7	III	3	mPSL pulse, CsA→ FK506, ATG	1700	33	+		EX	Encephalopathy	Dead, encephalopathy (day +253)
8	IV	3	mPSL pulse, ATG	2340	37	+	FFP	NE	VOD	Dead, VOD and liver failure (day +83)
9	III	2	mPSL pulse	1480	32	+	FK506/PSL reduced	EX		Dead, cGVHD and respiratory failure (day +1020)
10	II	1	mPSL 2 mg/kg with FK506	910	29	+	FK506/PSL reduced, FFP	EX	BOOP	Alive, day +2049
11	III	3	mPSL 2 mg/kg with FK506	2410	13	+	FK506/PSL reduced	EX		Dead, cGVHD and respiratory failure (day +1098)
12	III	2	mPSL 2 mg/kg with FK506	1200	10	+	FK506/PSL reduced, FFP	EX	Hemorrhagic cystitis	Dead, ischemic colitis (day +473)
13	II	0	mPSL 4 mg/kg with FK506	1000	21	+	FK506/PSL reduced	LD	VOD, hemorrhagic cystitis	Dead, VOD and liver failure (day +202)
14	II	0	mPSL 2 mg/kg with FK506	2595	34	+	FK506/PSL reduced, FFP	NE	Encephalopathy, hemorrhagic cystitis	Dead, multiple organ failure (day +88)
15	III	3	mPSL 2 mg/kg with FK506	3400	42	+	PSL reduced	None	VOD	Alive, day +1320
16	0	0	—	1750	57	+	FK506 reduced	None		Alive, day +1250

Patients 1–7 were retrospectively diagnosed as TMA mPSL = methyl-prednisolone; CsA = cyclosporinA; ATG = antithymocyte globulin; FK506 = tacrolimus; NE = not evaluable; EX = extensive type; LD = limited type; VOD = veno-occlusive disease; BOOP = bronchiolitis obliterans organizing pneumonia; PSL = prednisolone; FFP = fresh-frozen plasma.

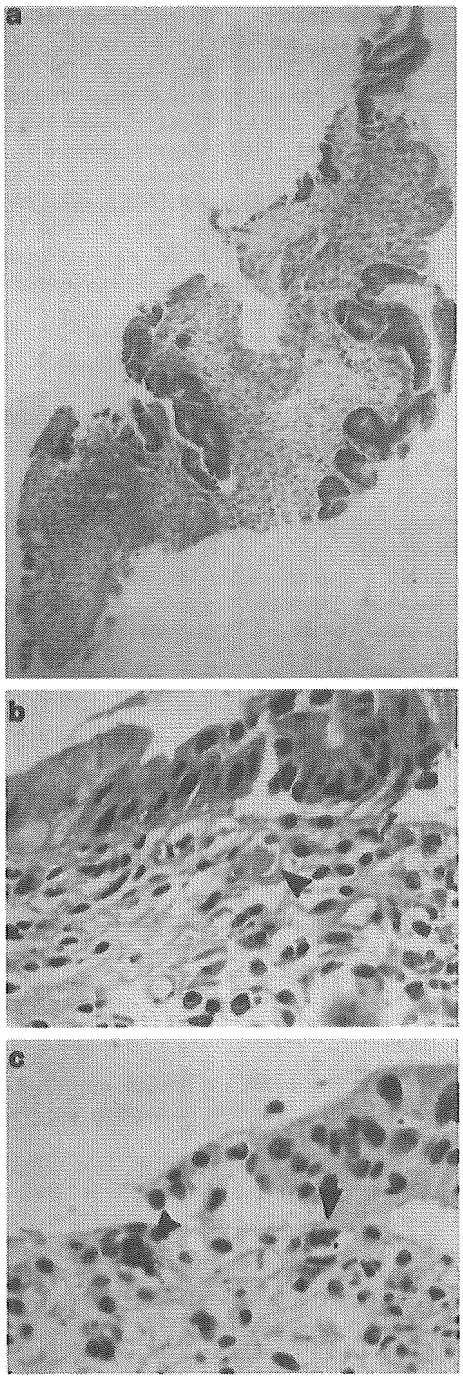

Figure 1 Pathologic features of the colon in colonoscopic biopsy specimens from patient 12. (**a**) Diffuse sloughing of epithelial cells and perivascular hemorrhages in the mucosa are seen (HE stain, × 100). (**b**) Thrombus (arrow) are recognized in the capillary, but no infiltration of lymphocytes are observed (HE stain, × 400). (**c**) Immunohistochemical staining with monoclonal antibody against CD41a (anti-GPIIbIIIa) shows platelets thrombi (arrows) in the capillaries.

GVHD. However, histological findings made differential diagnosis from gut GVHD possible. In addition, the areas of histologic hemorrhage provided evidence of the existence of capillary platelet thrombi that contained GPIIbIIIa(CD41a) and vWF, representing histologic evidence of gut microangiopathy. For two patients who did not meet the clinical criteria of TMA, the pathological diagnosis of TMA could be made by means of colonoscopic biopsies. These results emphasize the significance of histological examination for differential diagnosis of intestinal TMA from acute GVHD for patients with diarrhea after BMT.

Acute GVHD and intestinal infection are thought to be the most common causes of diarrhea after the resolution of regimen-related gut toxicity and produce similar clinical symptoms, including lower abdominal pain, profuse diarrhea and intestinal bleeding.[23–30] We found that the intestinal tract is a prevalent site for post-BMT TMA and that ischemic enterocolitis due to microangiopathy is another mimic of gut GVHD. Although the clinical symptoms of intestinal TMA are similar to those caused by gut GVHD, the distinction between intestinal TMA and gut GVHD is particularly important for clinical procedures. Thus, the involvement of TMA in the gut as one of the targets for acute GVHD clearly indicates the need for substantial revision of current strategies for the diagnosis and treatment of acute GVHD.

It has been reported that multifactorial TMA is associated with high mortality.[1,2] The patients in our study suffered from various complications such as VOD, encephalopathy and hemorrhagic cystitis, and 13 (81%) of the 16 patients died. TMA may affect a multiplicity of organs besides the intestinal tract. Severe diarrhea induces the loss of albumin and immunoglobulin, so that patients with diarrhea may have a higher incidence of life-threatening infection than those without.

The immunosuppressive drugs CsA and glucocorticoids (GCs) reportedly have adverse effects on endothelial cells and platelets.[31–36] CsA inhibits the ability of cultured human endothelial cells to produce prostacyclin (PGI_2) in response to arachidonic acid, thrombin or calcium ionophores.[31] Endothelial cell-dependent protein C activation decreases as a result of treatment with CsA,[32] and CsA nephrotoxicity causes an increase in plasma thrombomodulin levels as an indicator of vascular injury.[33] In the case of platelets, CsA directly increases the aggregation of normal platelets in response to adenosine diphosphate or epinephrine.[34] GCs also block endothelial cell production and the release of the vasodilators prostaglandin (PGE_2) and PGI_2.[35] The administration of GCs to thrombocytopenic rabbits resulted in a dose-dependent shortening of the bleeding time, which may be caused by the inhibition of endothelial PGI_2 production and consequent augmentation of platelet aggregation.[36] FK506 may also have similar effects, so that the prolonged administration of these agents may result in an increased risk of TMA. Indeed, it has been reported that CsA and FK506 are associated with TMA.[14,37] Our results showed that prompt tapering off of these immunosuppressants resulted in the resolution of the gut symptoms and long survival in some of these patients.

On the other hand, chronic GVHD is a common late complication of allogeneic BMT and worsens the post transplant prognosis.[38] Since two of our cases died of progressive chronic GVHD after the tapering off of immunosuppressive agents, careful attentions should be paid to the occurrence or progression of chronic GVHD after the reduction of immunosuppressants. An exact effect of the reduction of immunosuppressants for the treatment

of TMA should be determined in a prospective study with a substantially larger number of subjects.

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Feasibility of HLA-haploidentical hematopoietic stem cell transplantation between noninherited maternal antigen (NIMA)–mismatched family members linked with long-term fetomaternal microchimerism

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Based on the hypothesis that long-term fetomaternal microchimerism is associated with acquired immunologic hyporesponsiveness to noninherited maternal antigens (NIMAs) or inherited paternal antigens (IPAs), several groups have recently reported successful cases of non-T-cell–depleted hematopoietic stem cell transplantation (SCT) from HLA-haploidentical family members mismatched for NIMAs. In this study, we examined the outcomes of 35 patients with advanced hematologic malignancies who underwent HLA-2-antigen– or HLA-3-antigen–incompatible SCT from a microchimeric NIMA-mismatched donor. After standard-intensity or reduced-intensity preparative regimens, all patients had sustained hematopoietic recovery with tacrolimus-based graft-versus-host disease (GVHD) prophylaxis. Grade II/IV acute GVHD occurred in 19 (56%) of 34 evaluable patients, while extensive chronic GVHD developed in 13 (57%) of 23 patients who could be evaluated. Multivariate analysis demonstrated that NIMA mismatch in the GVH direction was associated with a lower risk of severe grade III-IV acute GVHD when compared with IPA mismatch ($P = .03$). Fifteen patients were alive and 14 of them were disease-free with a median follow-up of 20 (range, 8 to 37) months. These results indicate that T cell–replete SCT from an HLA-haploidentical NIMA-mismatched donor can offer durable remission with an acceptable risk of GVHD in selected patients with advanced hematologic malignancies who lack immediate access to a conventional stem cell source. (Blood. 2004;104: 3821-3828)

Introduction

The lack of donor availability has been a major limitation to the widespread use of allogeneic hematopoietic stem cell transplantation (SCT), which is a curative treatment for various hematologic malignancies, bone marrow failure syndromes, and genetic disorders. Despite the presence of an increasing pool of unrelated volunteer donor registries, many patients who need allogeneic SCT are not able to find a histocompatible donor because of the inheritance of a rare or private HLA haplotype. Currently, unrelated umbilical cord blood is validated as an alternative stem cell source allowing less HLA restriction for children with malignant diseases.[1] However, its application to adult recipients is still associated with a high probability of early nonrelapse mortality mostly due to delayed or unsuccessful engraftment,[2] although a few centers have reported encouraging results.[3-6]

Genetically HLA-haploidentical family members are more readily accessible to most of the patients who fail to find an HLA-compatible related or unrelated donor. However, transplants from such donors are limited by a number of historical barriers such as intractable graft-versus-host disease (GVHD) or graft failure.[7,8] In the past decade, extensive efforts have been made to overcome these problems, especially in transplantations from HLA-2-antigen– or HLA-3-antigen–mismatched donors; those include partial T-cell depletion combined with intensive immunosuppression,[9] megadose $CD34^+$ cell transplantation,[10] and ex vivo anergy induction.[11] Although these studies clearly became the leads to safer and more effective ways to undergo highly HLA-disparate transplantations, their introduction into routine clinical practice still awaits further validation.

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A complete list of the members of the Japanese Collaborative Study Group for NIMA (noninherited maternal antigen)-Complementary Haploidentical Stem Cell Transplantation appears in the "Appendix."

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An alternative way to overcome histocompatible barriers in transplantation medicine would be to identify "permissible" or "acceptable" HLA mismatches.[12] The concept that some mismatches are not harmful in terms of allograft acceptance was originally proposed from earlier observations that different HLA mismatches had different influences on survival rates of kidney allografts.[13,14] In this scenario, more attention should be focused on the relatively low immunogenicity of the noninherited maternal HLA antigens (NIMAs) to which one had been previously exposed in utero. In the late 1980s, Claas and colleagues in Leiden reported that about half of the patients who received multiple blood transfusions as adults exhibited reduced alloreactivity against NIMAs, while they had much higher reactivity against noninherited paternal antigens (NIPAs).[15] The clinical significance of this observation was later revived through 2 retrospective analyses showing the superior long-term survival rates in NIMA-mismatched kidney allografts, although the introduction of new immunosuppressive drugs appeared to obscure such effects.[16,17] Furthermore, another study performed by the International Bone Marrow Transplant Registry (IBMTR) also revealed that bone marrow transplants from NIMA-mismatched siblings were significantly associated with a lower incidence of grade II to IV acute GVHD (aGVHD) when compared with those from the other HLA-mismatched familial donors.[18] However, the true existence of such tolerogenic "NIMA effect" has still been the subject of debate due to lack of the knowledge of the underlying immunologic mechanisms.[19,20]

Recently, Andrassy et al have shown in a murine experimental model that more than half of the H-$2^{b/b}$ offspring that had experienced both oral and in utero exposure to noninherited H-2^{d} antigens of semiallogenic H-$2^{b/d}$ mothers could accept fully allogeneic H-$2^{d/d}$ vascularized heart allografts for a long time.[21] Importantly, they also demonstrated that the tolerant mice had higher levels of maternal cell microchimerism after their birth as compared with the nontolerant mice, suggesting the role of long-term maternal microchimerism for the induction and maintenance of NIMA-specific allotolerance. In this context, we previously found that more than two thirds of healthy adults have hematopoietic cell microchimerism presumed to be of maternal or fetal origin and proposed that the presence of such microchimerism might have beneficial effects on the transplantation outcome by reducing host-versus-graft (HVG) or graft-versus-host (GVH) alloreactivity not only against NIMAs but also against inherited paternal HLA antigens (IPAs) in the setting of maternal donation.[22,23] On the basis of this hypothesis, several transplantation centers in Japan have started clinical trials to test the feasibility of HLA-haploidentical allo-SCT from microchimeric NIMA-mismatched relatives without using either T-cell depletion or intensive posttransplantation immunosuppression. In these transplantations, the HLA haplotypes that are not shared between the donor and recipient always include at least one of the noninherited maternal haplotypes of their own, mimicking the condition that NIMA is complementarily selected as an acceptable mismatch (NIMA-"complementary" transplantation) (Figure 1). To evaluate the safety and efficacy of this novel principle of alternative donor selection, we performed a nationwide collective analysis of the clinical outcomes of T cell–replete allogeneic SCT for advanced hematologic malignancies from HLA-2-antigen– or HLA-3-antigen–incompatible (in the GVH direction) familial donors that were mismatched for NIMAs.

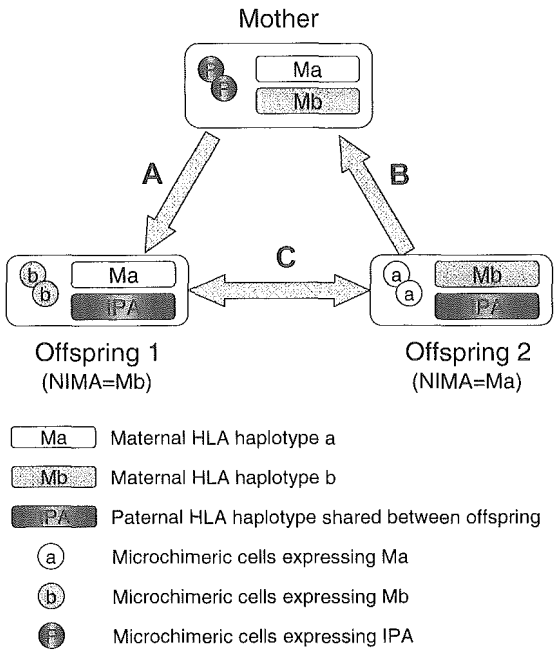

Figure 1. A scheme of 3 different types of NIMA-complementary HLA-haploidentical stem cell transplantation. (A) Stem cell transplantation (SCT) from mother to offspring: GVH reaction is directed against the inherited paternal HLA antigens (IPA), while HVG reaction is directed against the NIMAs of offspring 1 (Mb). (B) SCT from offspring to mother: GVH reaction is directed against the NIMAs of offspring 2 (Ma), and HVG reaction is directed against IPAs. (C) SCT between NIMA-mismatched siblings who shared the inherited paternal HLA haplotype: These siblings are bidirectionally mismatched for NIMAs in both the GVH and HVG directions.

Patients and methods

Study patients and data collection

This registry study included a total of 35 patients with advanced hematologic malignancies who underwent non-T-cell–depleted SCT from an HLA-haploidentical NIMA-mismatched family member at 16 transplantation centers affiliated with the Japan Society for Hematopoietic Cell Transplantation (JSHCT)[24] between May 2000 and June 2003. All the patients, the donors, or their guardians provided written informed consents on the protocols for the NIMA-complementary haploidentical transplantation, which were approved by the institutional review boards at participating centers. The detailed clinical course of 8 of these patients was previously reported in several papers.[25-29] The eligibility criteria for the study were as follows: (1) having advanced leukemia or lymphoma; (2) receiving T cell–replete bone marrow and/or peripheral blood stem cell (PBSC) grafts mismatched for 2 or 3 HLA-A, -B, or -DR antigens in the GVH direction harvested from mother, offspring (to mother), or an NIMA-mismatched sibling who was shown to have recipient-specific microchimerism presumed to be of fetal or maternal origin; (3) receiving tacrolimus-based GVHD prophylaxis. Using a standardized questionnaire form, participating centers were requested to consecutively report data with respect to the patient/donor characteristics and clinical outcomes in terms of neutrophil recovery, platelet recovery, aGVHD, chronic GVHD (cGVHD), graft failure, relapse, and survival after transplantation to the office of the Japanese Collaborative Study Group for NIMA-Complementary Haploidentical Stem Cell Transplantation (Department of Hematology/Oncology, Kyoto University Hospital, Japan). The reported data sets including the clinical severity of aGVHD and cGVHD were thoroughly reviewed by 2 independent physicians. All the patients were longitudinally followed until February 29, 2004.