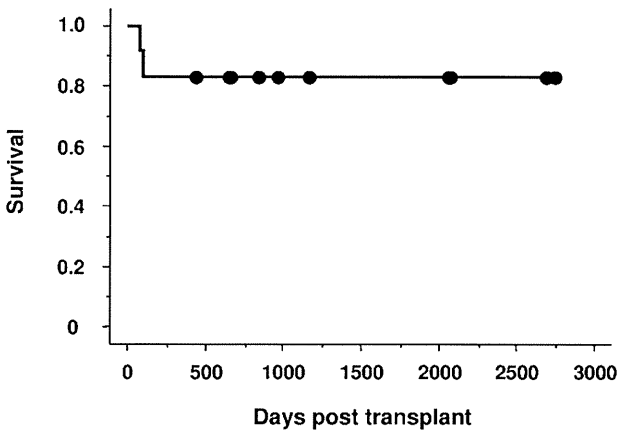

Figure 1. Survival of 12 patients with SAA undergoing unrelated cord blood transplantation.

patients with anti-HLA antibodies, when the specificity corresponding to mismatched antigen in UCB graft, showed significantly lower neutrophil or platelet recovery than those with antibody-negative or -positive but not corresponding to UCB graft.[17] Although the observations may differ from that of diverse populations and warrants further investigation, if possible, the use of a UCB unit with corresponding HLA antibodies in the recipient should be avoided.

Three-year survival in the studied patients was 83.3%. In addition to high rate of engraftment, the low risk of severe GVHD might contribute to high survival rate with good quality of life, and seems to be one of the important advantages of using a UCB unit for SAA patients. The other advantage of the use of UCB units is rapid availability. In the present study, 2 patients with fulminant type could be rescued by urgent hematopoietic stem cell transplantation using UCB units. More than 90% of recipients can find a suitable UCB unit in Japan; thus, UCB expands the chance to receive transplantation for those who need it urgently.

In conclusion, this retrospective study strongly suggests the feasibility and effectiveness of RI-UCBT for adult SAA patients. RI-UCBT may become a viable therapeutic option for those who lack suitable HLA-matched donors and who fail or relapse after immunosuppressive therapy. Although our results should be interpreted with caution because of the small number of patients and still short follow-up duration, we think that RI-UCBT with the conditioning regimen presented here deserves further evaluation in a prospective trial, hopefully in a multicenter setting.

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Authorship

Contribution: H.Y. and D.K. performed transplantation, analyzed extracted data, and contributed to writing the paper; A.Y. reviewed histopathologic sections; H.Y. and N.M. performed statistical analysis; N.U., K. Izutsu, and S. Taniguchi reviewed study design and methods; and K. Ishiwata, H.A., S. Takagi, M.T., N.N., Y.A.-M., K.M., A.W., and S.M. performed transplantation and contributed to writing the paper.

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LETTER TO THE EDITOR

What is the upper age limit for performing allo-SCT? Cord blood transplantation for an 82-year-old patient with AML

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Since morbidity and mortality associated with hematologic malignant diseases in elderly patients is higher than that in younger patients,[1] elderly patients are less likely to be candidates for allo-SCT, due to the facts that they are more likely to have comorbid organ conditions, either clinically or subclinically, which results in a higher rate of procedure-related mortality,[2] and that they are less likely to have HLA-matched related donors available, as siblings also tend to be elderly.

The development of reduced-intensity (RI) conditioning for transplants, which results in less toxicity and depends largely on GVL effects rather than high-dose therapy to eliminate leukemic cells, has been shown to allow elderly patients to undergo allo-SCT.[3–5] The use of umbilical cord blood transplantation (UCBT) for adults has been increasing due to the potential advantage of rapid availability and the lower risk of GVHD, thus permitting less stringent HLA matching.[4,5] RI-UCBT for adults, mostly elderly patients, has been increasingly reported and shown to be applicable.[6,7] However, there has been no clear description on the upper age limit of receiving allo-SCT, and it varies among institutes at this moment. We report here an 82-year-old man with refractory AML who had successfully treated with RI-UCBT.

The patient was diagnosed as AML (M5b) with adverse risk karyotype (46, XY, −7, +8) and complicated with disseminated intravascular coagulation (DIC). Although DIC was resolved soon after remission induction therapy consisted of idarubicin and cytarabine, and the patient achieved hematological remission, the disease subsequently progressed with lung infiltration and systemic skin tumor formation (Figure 1a). Immunohistochemical analysis of skin tumor showed positive for CD45, myeloperoxidase, and CD68 consistent with leukemic cell infiltration. Skin and lung infiltration was refractory to following high-dose Ara-C-containing chemotherapy. At 4 months after diagnosis of AML, following careful discussion and consent among the patient, his family and transplant staff, he received an RI-UCBT using two antigen- and three allele-mismatched CB in August 2007. His Eastern Cooperative Oncology Group (ECOG) performance status was 2, and HCT-CI score was 1. The preparative regimen consisted of i.v. fludarabine 25 mg/m^2 daily for 5 days (total dose 125 mg/m^2), i.v. melphalan 40 mg/m^2 daily for 2 days (total dose 80 mg/m^2) and 4 Gy of TBI fractionated by 2. GVHD prophylaxis consisted of tacrolimus by continuous infusion and 15 mg/kg twice daily of oral mycophenolate mofetil from day −1. CB unit contained 2.5×10^7 per kg of total nucleated cells and 0.98×10^5 per kg of CD34+ cells before cryopreservation. G-CSF 300 μg/m^2 was administered from day 1 until neutrophil engraftment. On day 14, the patient developed erythema, fever (39 °C) and diarrhea, and was diagnosed as having preengraftment immune reactions (PIR).[8] The symptoms disappeared immediately after initiation of methylprednisolone 0.5 mg/kg for 3 days. There was no episode of bacterial infection during neutropenia. ANC recovered to 0.5×10^9 per liter on day 25, and platelet count reached 2.0×10^9 per liter on day 64. Complete donor-cell chimerism was confirmed on day 27 by BM analysis using short tandem repeat-PCR method. Human herpesvirus-6 limbic encephalitis developed on day 17, which was successfully managed with foscarnet. The regimen-related toxicities observed were mucositis (grade 2), nausea (grade 2), renal dysfunction (grade 2) and diarrhea (grade 1), according to the National Cancer Institute Common Toxicity Criteria version 3.0. Acute GVHD of grade III (gut: stage 2) on day 46 was observed, but successfully managed with oral beclomethasone dipropionate. He finally achieved CR in BM, and his lung lesion and skin tumors also disappeared (Figure 1b). He was discharged from hospital on day 123 after RI-UCBT. To our surprise, his level of performance status got improved thereafter, almost as score 1 measured by ECOG PS scoring system, and returned to his work in 1 month after discharge. In the meantime, chronic GVHD of limited type developed, which was managed without treatment. One year after RI-UCBT, unfortunately, his disease relapsed and he died from disease progression 1 month later.

This remarkable case told us two important issues. First, some, may be not all, patients older than 80 years still can tolerate RI-UCBT. TRM has been shown to be correlated with several factors including age, or more comprehensively, the number of coexisting comorbidities.[9] According to our previous report, those older than 54 years showed cumulative incidence of TRM reaching to approximately 50%, and most of TRM occurred early period post-UCBT.[10] This patient had also faced life-threatening events, such as PIR or viral encephalitis, and was successfully managed by corticosteroid and foscarnet. In allo-SCT settings, there are always several factors that cannot be modulated intentionally, and there may have been good coincidences for him to reach this successful outcome. Nevertheless, this case strongly claims higher age should not be the single determinant of not performing allo-SCT. Second, the most powerful antileukemic activity was observed with RI-UCBT. Although, the patient had finally disease relapse, it was obvious that only RI-UCBT sufficiently suppressed leukemic cells and gave him a

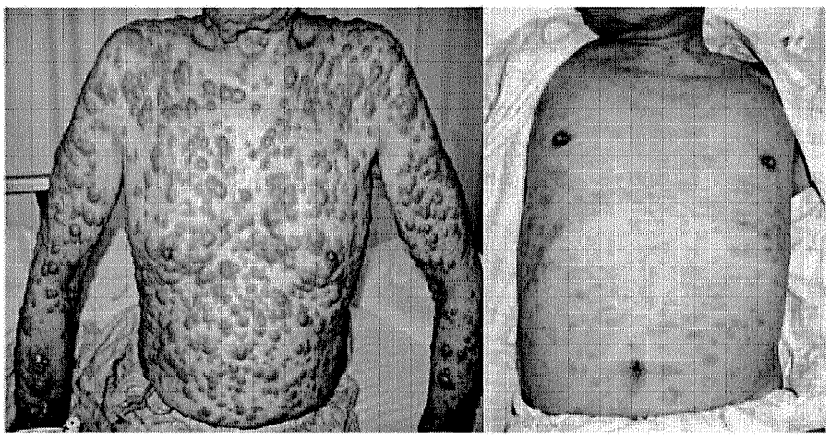

Figure 1 Skin tumors covering whole body of the patient just before RI-UCBT (**a**). Skin tumors of the patient had disappeared in 90 days after RI-UCBT (**b**).

sustained CR so that he had enough time to return to his job. Although CB has been shown to have functionally immature immune cells, it showed its extremely powerful anti-leukemic activity even from the early period post transplant, as the patient's skin lesion had never disappeared during induction chemotherapy including high-dose Ara-C.

Whether the clinical course of this case can be applicable to all aged patients or this is exceptional case needs to be investigated carefully. The indication of allo-SCT for those who are elderly has to be determined individually with extremely careful and repeated discussion with patients, families and transplant staff. Nevertheless, the indication of allo-SCT should not be determined by age as a sole factor. Otherwise, elderly patients may lose chance of cure or good disease control, by not performing toxic yet powerful treatment, such as transplant.

Conflict of interest

The authors declare no conflict of interest.

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Case Report

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An Epstein-Barr Virus-Associated Leukemic Lymphoma in a Patient Treated with Rabbit Antithymocyte Globulin and Cyclosporine for Hepatitis-Associated Aplastic Anemia

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Key Words

Antithymocyte globulin · Aplastic anemia · Diffuse large B-cell lymphoma · Epstein-Barr virus · Lymphoproliferative disorder

Abstract

Lymphoproliferative disorders (LPDs) are generally caused by uncontrolled B-cell proliferation induced by the Epstein-Barr virus (EBV) in the setting of impaired EBV-specific T-cell immunity, particularly when there is pharmacological immunosuppression including antithymocyte globulin. We herein present an unusual case of EBV associated with LPD (EBV-LPD) in which LPD occurred 3 weeks after the use of rabbit antithymocyte globulin administered for severe hepatitis-associated aplastic anemia; the patient died of fulminant leukemic lymphoma 5 days after the onset. We also review the pertinent literature on EBV-LPD after immunosuppressive therapy and document the efficacy of EBV viral load monitoring and the need for preemptive therapy.

Introduction

Epstein-Barr virus (EBV)-associated lymphoproliferative disorders (LPDs) are becoming a serious problem with a recent increase in the number of patients with immunodeficiency. In particular, patients who have undergone allogeneic hematopoietic stem cell transplantation (HSCT) are predisposed to EBV infection or reactivation and development of EBV-related diseases [1]. EBV monitoring is generally recommended for high-risk patients such as HSCT recipients of human leukocyte antigen mismatched donors and patients receiving antithymocyte globulin (ATG) after HSCT. Early detection of EBV reactivation would make it possible to offer preemptive therapy with rituximab if necessary, thus preventing the proliferation of EBV-infected B cells and the evolution of B-cell lymphoma.

Acquired severe aplastic anemia (SAA) is a rare disease defined by peripheral blood pancytopenia associated with hypocellularity of the bone marrow [2]. Because bone marrow failure is thought to result from an immune-mediated mechanism, immunosuppressive therapy (IST) is the treatment of choice in patients without a suitable donor for HSCT. IST including ATG and cyclosporine A (CsA) is the most effective treatment for SAA [3]. Several studies have shown that the use of ATG increases the fre-

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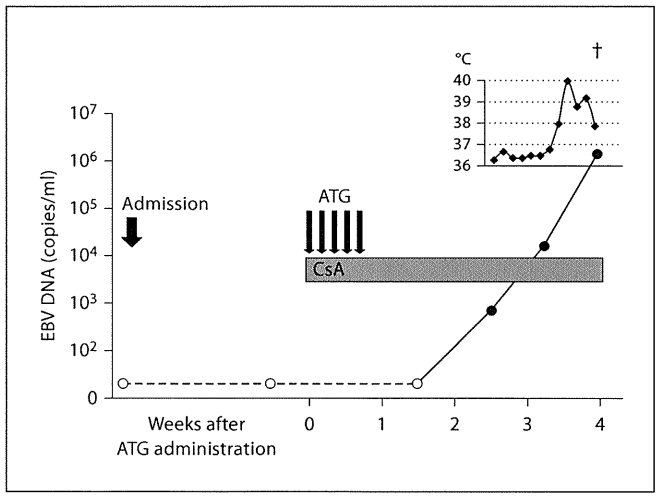

Fig. 1. Changes in plasma EBV DNA and the patient's body temperature. Open and closed circles represent EBV-negative and -positive states, respectively. Increase in EBV DNA was observed 2 weeks after administration of ATG.

quency of EBV reactivation and the risk of EBV-LPD [4]. However, this risk has not yet been sufficiently documented in patients treated with ATG for SAA. It is also unclear whether EBV viral load monitoring during IST for SAA and subsequent preemptive therapy are beneficial [5, 6].

We herein report an unusual case of fulminant EBV-LPD that occurred in a patient as a form of leukemic lymphoma 3 weeks after the first administration of rabbit ATG and CsA for hepatitis-associated AA.

Case Report

A 54-year-old male presented to the hospital with a 3-week history of general malaise and loss of appetite. He showed jaundice and had severely deranged liver function tests, with a total bilirubin of 5.5 mg/dl (normal range 0.3–1.2, direct fraction 3.1), alkaline phosphatase 536 IU/l (normal range 115–359), aspartate aminotransferase 1,021 IU/l (normal range 13–33), alanine aminotransferase 2,718 IU/l (normal range 8–42) and γ-glutaryl transpeptidase 288 IU/l (normal range 10–47). His blood tests showed a platelet count of 12 × 10^9/l, a hemoglobin level of 13.1 g/dl and a white blood cell (WBC) count of 1.4 × 10^9/l, with 0.7 × 10^9/l neutrophils. The absolute counts of CD4- and CD8-positive T cells were 0.2 and 0.1 × 10^9/l, respectively. A blood film showed leukopenia and thrombocytopenia, with no abnormal morphology. There was no history of recent travel, blood transfusions, use of medications or excess alcohol consumption. Subsequent investigations showed no evidence of a viral etiology. Hepatitis A virus immunoglobulin M (IgM), hepatitis B virus antigen, hepatitis B core IgM, hepatitis C virus antibody, hepatitis C RNA PCR, hepatitis E virus IgM and IgG were all negative. The hepatitis B surface antibody was positive, but hepatitis B virus PCR was negative. The cytomegalovirus IgG was positive and cytomegalovirus IgM was weakly positive. His anti-EBV antibody titers were viral capsid antigen IgG positive, viral capsid antigen IgM negative, early antigen negative, and EBV-determined nuclear antigen positive. Parvovirus B19 IgM and IgG serology were negative. The patient was also negative for HIV antibodies. The bone marrow was severely hypocellular, which was consistent with AA. Immunophenotyping of bone marrow cells was normal, and there was no evidence of paroxysmal nocturnal hemoglobinuria. A liver biopsy was not performed due to the presence of severe thrombocytopenia. We diagnosed him with hepatitis-associated AA. ATG therapy was put on hold until his liver function tests improved, but his pancytopenia progressed without normalization of his jaundice. Rabbit ATG was started 3 weeks after admission at a dose of 3.75 mg/kg on days 1–5, and CsA at a dose of 3 mg/kg with methylprednisolone, which resulted in a rapid improvement in his liver function. He was further treated with prednisolone for prophylaxis of serum sickness, with normalization of liver function tests, but his hematological data still showed pancytopenia.

Three weeks after the administration of ATG, the patient developed a persistent high fever, which was refractory to antibiotics and antifungal agents. Moreover, his liver function tests worsened, including a total bilirubin of 1.9 mg/dl, aspartate aminotransferase 408 IU/l, alanine aminotransferase 577 IU/l, γ-glutaryl transpeptidase 779 IU/l, and alkaline phosphatase 848 IU/l. Blood tests showed a platelet count of 15 × 10^9/l, a hemoglobin level of 9.2 g/dl and a WBC count of 7.5 × 10^9/l. A peripheral blood smear revealed an increased number of lymphocytes (2.0 × 10^9/l), but no apparent hemophagocytic findings. The phenotype of the atypical lymphocytes was CD3–, CD10–, CD19+, CD20+ and IgG light chain lambda+. The serum ferritin level (199,770 ng/ml) was also markedly elevated. Based on these clinical signs, laboratory data and the use of ATG, EBV-LPD was highly suspected. The administration of rituximab was considered, but severe metabolic acidosis and cardiorespiratory failure developed the evening after EBV-LPD was diagnosed and the patient died of LPD on the following day.

The belated results of the peripheral blood EBV DNA (3.3 × 10^6 copies/10^6 WBC) and the pathological examination from the liver biopsy (demonstrating an increase in CD20+ lymphocytes that were positive for EBV-encoded mRNA by in situ hybridization) confirmed the diagnosis of EBV-associated diffuse large B-cell lymphoma. Infiltration of the lymphoma was also detected in his bone marrow. We analyzed the patient's peripheral plasma EBV DNA retrospectively. His plasma showed an elevation of the EBV viral load to 700 copies/ml for the first time at 7 days before the onset of pyrexia, and the EBV viral load rapidly increased within 5 days (fig. 1).

Discussion

We herein describe the occurrence of fulminant EBV-LPD that was diagnosed following a sharp increase in the atypical B-cell count in peripheral blood 3 weeks after IST for hepatitis-associated AA. The patient died of car-

Table 1. Reports of LPD after IST for AA

References	Age, years	sex	Type of LPD	Immunosuppression
Dorr et al. [8], 1996	17	female	lymphoma	ATG, CsA
Sarangi et al. [9], 1999	22	male	T-ALL	CsA
Takeuchi et al. [10], 2000	54	female	B-ALL	CsA, PSL=, others
Hirose et al. [11], 2001	24	male	T-ALL	CsA
Calistri et al. [12], 2006	38	male	infectious mononucleosis	rATG, CsA, M-PSL → hATG
Wondergem et al. [6], 2008	42	female	EBV(+) DLBCL	hATG, CsA → rATG, CsA
Suzuki et al. [13], 2009	63	female	EBV(–) DLBCL	ATG, CsA
Viola et al. [14], 2011	55	male	EBV(+) plasma cell hyperplasia	hATG
Our case	54	male	EBV(+) DLBCL	rATG, CsA, PSL

T-ALL = T-cell acute lymphoblastic leukemia; B-ALL = B-cell acute lymphoblastic leukemia; PSL = prednisolone; rATG = rabbit ATG; M-PSL = methylprednisolone; hATG = horse ATG; DLBCL = diffuse large B-cell lymphoma.

diorespiratory failure associated with severe lactic acidosis due to rapidly progressive lymphoma. Lactic acidosis in association with hematologic malignancies normally shows an extremely poor prognosis [7].

It is well documented that EBV is an important complication of prolonged immunodeficiency. All patients who have a limited number of circulating T cells and retain B cells are at risk of developing EBV reactivation, as the interplay among EBV replication, latency and immune control is not as balanced as in the healthy host. When there is pharmacological immunosuppression, EBV reactivation can lead to LPD because T-cell function is severely impaired and B cells can evade the T-cell attack and expand. This is particularly common in patients undergoing HSCT with an ATG-containing conditioning regimen [4].

Treatment with ATG combined with CsA is the standard therapeutic approach to SAA. Scheinberg et al. [5] showed the risk of EBV disease in patients treated with ATG for AA to be low. In their study of 78 patients with SAA who had received 4 different immunosuppressive regimens, including ATG, even though EBV reactivation occurred in most patients, none developed symptomatic EBV-LPD. To determine the incidence of clinically significant EBV-LPD, we searched Medline for published articles about LPD after IST for AA. As shown in table 1, a total of 8 cases of LPD that occurred after IST have been reported [6, 8–14].

Viola et al. [14] recently reported a patient who developed LPD 1 month after the use of horse ATG for the treatment of SAA. Although the interval between ATG therapy and the onset of LPD in this case was short, similar to our case, he had received chemotherapy and autologous HSCT for the treatment of non-Hodgkin's lymphoma 3 years prior to the ATG therapy, both of which may have predisposed the patient to develop LPD.

Wondergem et al. [6] described a patient who received a higher dose of rabbit ATG for SAA after failing to respond to horse ATG. The patient then developed life-threatening EBV-related lymphoma. In our patient, LPD occurred after his first ATG therapy. To the best of our knowledge, this is the first report of an EBV-related diffuse large B-cell lymphoma in a patient treated with a single course of rabbit ATG.

Recently, the viral load has been shown to be a significant predictor of EBV-related post-transplant LPD [15, 16], and early treatment with an anti-CD20 antibody is recommended as preemptive therapy in patients undergoing an alternative donor transplant [1]. Because of the rapid clinical course of EBV-LPD, immediate treatment is crucial to reduce mortality [6]. No lymphocytosis or pyrexia was observed when the EBV DNA level began to increase in our patient (fig. 1). Preemptive therapy with rituximab may improve the treatment outcome of EBV-LPD, not only after HSCT [17], but also after IST for SAA. However, the EBV copy number in the plasma of the current patient at day 19 of ATG therapy, 1 week before the onset of pyrexia, was 700 copies/ml, but it increased by more than 20 fold on day 24 (16,000 copies/ml). Therefore, once-a-week screening of EBV would have been useless for this patient. The prompt examination of blood for EBV copy number in response to clinical signs such as pyrexia may be more practical than surveillance for appropriately starting rituximab. The preventive administration of rituximab would be a possible option for AA patients with a high risk of developing EBV-LPD.

Several risk factors for susceptibility to EBV-related lymphoma have been identified. Dierksheide et al. [18] showed that an interferon-γ polymorphism affects a likelihood of EBV reactivation. Genotyping of the interferon-γ gene may be useful for identifying patients at greater risk of developing EBV-LPD. The percentage of CD4+ T cells in hepatitis-associated AA patients is reported to be significantly lower than that in non-hepatitis-associated AA patients [19]. Therefore, the presence of hepatitis-associated AA may have predisposed our patient to developing EBV-LPD.

The short time interval between ATG treatment and diagnosis and the fulminant course of EBV-LPD in our case and in the case reported by Viola et al. [14] may be related to the profound immunosuppressive state associated with hepatitis and the precedent chemotherapy in these cases.

The present case indicates that fulminant EBV-associated lymphoma can occur in patients with AA even after a single course of rabbit ATG therapy. Close monitoring of the EBV viral load is therefore a prerequisite for rabbit ATG therapy of AA.

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ORIGINAL ARTICLE

Allogeneic hematopoietic stem cell transplantation for acute myeloid leukemia with t(6;9)(p23;q34) dramatically improves the patient prognosis: a matched-pair analysis

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Acute myeloid leukemia (AML) with t(6;9)(p23;q34) is well known to have a poor prognosis treated with chemotherapy and autotransplantation. The presence of this karyotype is an indicator for allogeneic hematopoietic stem cell transplantation (HSCT); however, the impact of t(6;9)(p23;q34) on the HSCT outcome remains unclear. We conducted a matched-pair analysis of *de novo* AML patients with and without t(6;9)(p23;q34) using data obtained from the Japanese HSCT data registry. A total of 57 patients with t(6;9)(p23;q34) received transplants between 1996 and 2007, and 171 of 2056 normal karyotype patients matched for age, disease status at HSCT and graft source were selected. The overall survival, disease-free survival, cumulative incidence of relapse and the non-relapse mortality in t(6;9)(p23;q34) patients were comparable to those for normal karyotype patients. A univariate analysis showed that t(6;9)(p23;q34) had no significant impact on the overall survival. These findings suggest that allogeneic HSCT may overcome the unfavorable impact of t(6;9)(p23;q34) as an independent prognostic factor.
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Keywords: allogeneic hematopoietic stem cell transplantation; acute myeloid leukemia; unfavorable cytogenetic risk; t(6;9)(p23;q34)

Introduction

Acute myeloid leukemia (AML) is a hematological malignancy resulting from the proliferation of leukemic stem cells. Because of the resistance of leukemic stem cells to chemotherapy,[1] long-term survival is generally seen in only 50% of patients treated with chemotherapy alone. Therefore, allogeneic stem cell transplantation (HSCT) is often considered as a curative treatment option.[2] AML is the most common indication for HSCT in North America and in Japan, but fatal transplant-related adverse events are difficult to avoid, despite the improvements in supportive treatment in recent years. Therefore, treatment of AML is hard to standardize, and the attending physician must make a decision on a case-by-case basis, weighing the advantages and disadvantages of HSCT.

The results of previous large clinical trials have indicated that abnormalities of the chromosomal karyotype are considered to be one of the most powerful factors to predict the patient prognosis.[3,4] AML with the unfavorable cytogenetic risk group, such as a partial deletion of the long arm of chromosome 7 (del (7q)), monosomy of chromosome 7 (−7) or with a complex karyotype is considered to be a good indication for HSCT, even during the first remission, because of the high cytogenetic risk associated with chemotherapy and the beneficial outcome that can be achieved by HSCT.[5–8]

The translocation of chromosome (6;9)(p23;q34) forming the *DEK/NUP214* fusion mRNA is observed in ~1% of AML cases.[9] The characteristics of AML with t(6;9)(p23;q34) are known to include development at a younger age,[10] resistance to chemotherapy and a very poor prognosis.[9] Therefore, the presence of this karyotype in AML patients is an indication for HSCT; however, the impact of t(6;9)(p23;q34) on the outcome of HSCT remains unclear because of the rarity of this entity. We conducted a retrospective study to examine the outcomes of HSCT in AML patients with t(6;9)(p23;q34) using the data from the Japan Society for Hematopoietic Cell Transplantation Data Registry.

Materials and methods

Study population

Clinical data were collected from the databases of the Japan Society for Hematopoietic Cell Transplantation and the Japan Cord Blood Bank Network using a standardized report form. Follow-up reports were submitted at 100 days, 1 year and annually after HSCT. Patients with *de novo* AML aged 15 years or older at the time of first HSCT and who received the transplant between January 1996 and December 2007 were extracted from the databases. We compared the clinical features and the outcomes among the patients with t(6;9)(p23;q34) and the patients with a normal karyotype in G-band staining. Cytogenetic data were analyzed according to the Southwestern Oncology Group criteria in each institution[7] instead of by central review. We selected patient pairs with t(6;9)(p23;q34)

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and the normal karyotype using an optimal matching method with the following three matching factors: recipient age, disease status at HSCT and graft source. This study was approved by the Committee for Nationwide Survey Data Management of the Japan Society for Hematopoietic Cell Transplantation. Written informed consent was obtained in accordance with the Declaration of Helsinki.

Statistical analysis

The overall survival (OS) was defined as the number of days from HSCT until death from any cause. Disease relapse was defined as the number of days from HSCT to relapse of the underlying disease. Non-relapse mortality was defined as death without relapse. Any patient who was alive at the last-follow-up date was censored. All statistical analyses were performed using the R version 2.13.0 software program (R Foundation for Statistical Computing; http://www.r-project.org). Probabilities and times-to-events were compared between the two groups using the Mantel–Haenszel method and stratified Cox's proportional hazard modeling, respectively. The cumulative incidences of non-relapse mortality and relapse were calculated considering each other event as a competing risk, and were compared using the stratified Grey test.[11] *P* values were two sided, and outcomes were considered to be significant when $P \leqslant 0.05$.

Results

Patients' characteristics

A total of 2577 AML cases met the inclusion criteria. The number of cases with t(6;9)(p23;q34) and a normal karyotype was 57 and 2056, respectively; and 171 patients with the normal karyotype were selected for matched-pair analysis by a 1:3 matching ratio. The characteristics of the patients are shown in Table 1; there were no statistically significant differences between the t(6;9)(p23;q34) patients and the normal karyotype patients except the use of total body irradiation as a preconditioning regimen.

Survival, relapse and non-relapse mortality

The probability of OS in the patients with t(6;9)(p23;q34) was as good as that for patients with a normal karyotype (the probability of 5-year OS in t(6;9)(p23;q34) and normal karyotype patients was 45 and 40%, respectively; Figure 1a). When the t(6;9)(p23;q34) patients and the normal karyotype patients were further categorized according to the disease status at HSCT, the OS of the t(6;9)(p23;q34) patients and the normal karyotype patients were comparable in both the complete remission (CR) at HSCT patients and the non-CR at HSCT patients (Figure 1b). The probability of disease-free survival in these patients was also not significantly different (the probability of 5-year disease-free survival in patients with t(6;9)(p23;q34) and the normal karyotype was 42 and 33%, respectively; Figure 1c). The cumulative incidence of relapse (Figure 2a) and the non-relapse mortality (Figure 2b) in t(6;9)(p23;q34) patients were also comparable to those for normal karyotype patients (the 5-year cumulative incidence was 42% in t(6;9)(p23;q34) patients and 45% in normal karyotype patients for relapse ($P=0.34$) and 16 and 22% ($P=0.85$) for non-relapse mortality). The prognostic factors affecting OS revealed that there were no significant differences related to karyotype, gender, gender mismatch between donor and recipient, human leukocyte antigen disparity, recipient cytomegalovirus serostatus and use of total body irradiation for the preconditioning regimen by the univariate analyses (Table 2).

Table 1 Patient characteristics

	t(6;9)(p23;q34)	*Normal karyotype*	P-*value*
Age			
15–24	14	42	0.999
25–34	14	45	
35–44	20	58	
45–54	7	20	
55–64	2	6	
Gender			
Male	34	97	0.758
Female	23	74	
Disease status at HSCT			
CR1 or CR2	29	87	1.0
Not in remission	28	84	
Preconditioning regimen, TBI			
No	21	33	0.0102
Yes	33	131	
Donor			
Related	26	78	1.0
Unrelated bone marrow	18	54	
Unrelated cord blood	13	39	
Number of HLA mismatch			
0	24	47	0.379
1	5	23	
2	10	27	
3	0	2	

Abbreviations: CR, complete remission; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; TBI, total body irradiation.

Discussion

Previous reports have confirmed the negative impact of t(6;9)(p23;q34) on the outcome after standard-dose chemotherapy and high-dose therapy with autologous stem cell transplantation in patients with AML.[9,10] The current matched-pair analysis of the nationwide survey demonstrated that the OS and the non-relapse mortality, as well as the relapse rate, were independent of the presence of t(6;9)(p23;q34) in allogeneic HSCT recipients, thus suggesting that allogeneic HSCT may be able to overcome the unfavorable effect of t(6;9)(p23;q34) in AML patients.

However, it is difficult to draw any firm conclusions regarding the results of the present analysis owing to the small number of patients in the matched-pairs subsets. These findings require confirmation in larger studies specifically in examining the impact of t(6;9)(p23;q34) status. Nevertheless, the suggestion that allogeneic HSCT appears to overcome the adverse survival impact of t(6;9)(p23;q34) is supported by other studies.[12,13] In a EBMT study of AML patients with t(6;9)(p23;q34), allogeneic HSCT produced responses that were independent of t(6;9)(p23;q34), and the 3-year OS of patients with t(6;9)(p23;q34) was as high as 51 ± 7%, comparable to AML patients with the normal karyotype.[13] Also, the incidence of relapse following allogeneic HSCT appeared to be similar in patients with t(6;9)(p23;q34) compared with those without

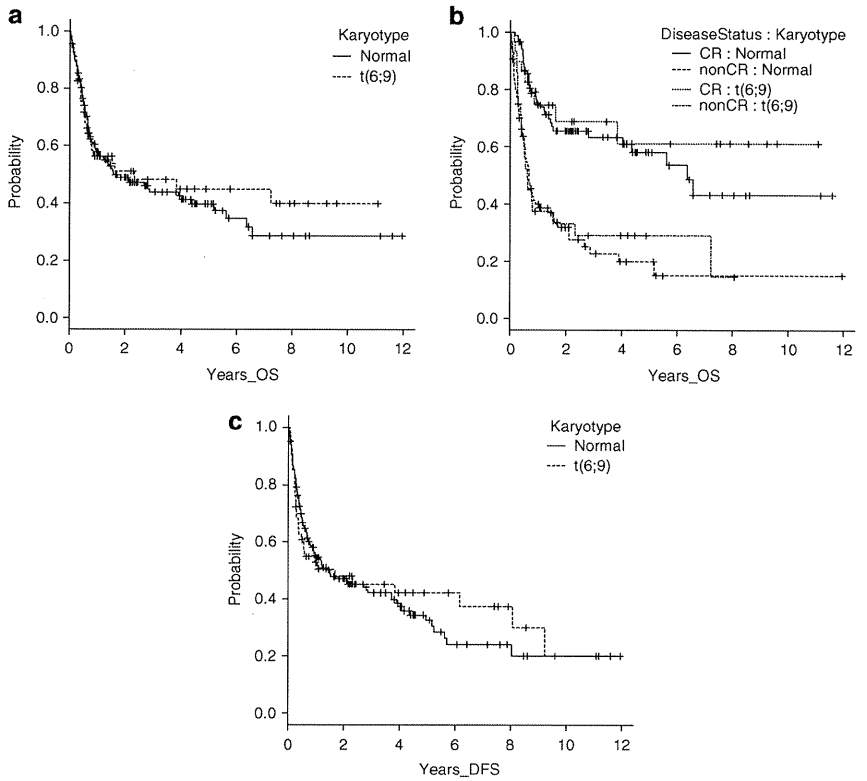

Figure 1 Survival of the patients. (**a**) The OS of the patients stratified by cytogenetics. Solid line, normal karyotype patients; dotted line, t(6;9) patients. (**b**) The OS of the patients grouped according to their disease status at transplantation. Solid line, normal karyotype patients in CR at HSCT; dashed line, normal karyotype patients in non-CR at HSCT; dotted line, t(6;9) patients in CR at HSCT; chain line, t(6;9) patients in non-CR at HSCT. (**c**) The disease-free survival of the patients. Solid line, normal karyotype patients; dotted line, t(6;9) patients.

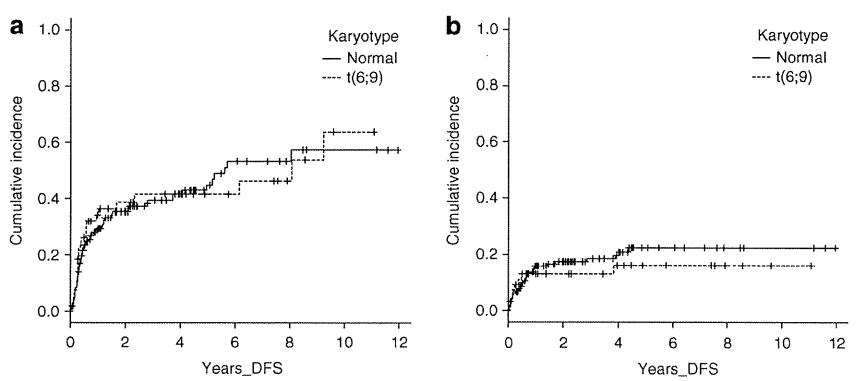

Figure 2 Cumulative incidence of events after transplantation stratified by cytogenetics. (**a**) The cumulative incidence of relapse of the patients. (**b**) The cumulative incidence of non-relapse mortality of the patients. Solid line, normal karyotype patients; dotted line, t(6;9) patients.

Table 2 Prognostic factors affecting overall survival

	Risk factor	*Hazard ratio*	*95% CI*	P-*value*
Karyotype	t(6;9)	1.07	0.66–1.74	0.79
Gender	Male	1.06	0.64–1.73	0.83
Gender mismatch	Female to male	1.41	0.74–2.68	0.29
HLA compatibility	Mismatch	0.98	0.57–1.75	0.94
Recipient CMV	Positive	0.27	0.028–2.70	0.27
Donor CMV	Positive	1.51	0.61–3.78	0.37
TBI	Yes	1.47	0.75–2.90	0.26

Abbreviations: CMV, cytomegalovirus; HLA, human leukocyte antigen; TBI, total body irradiation.

t(6;9)(p23;q34). However, the EBMT study made it somewhat difficult to determine whether HSCT would lead to a good outcome, because 87% of the patients were transplanted while in CR, whereas only 29 of 57 (51%) patients in our study received HSCT in CR, which is a more clinically relevant expectation, as a CR is difficult to achieve in these patients.

In conclusion, the current study showed that AML patients with t(6;9)(p23;q34) can be expected to have a post-transplant survival comparable to patients with a normal karyotype, thereby supporting the opinion that they are good candidates for HSCT.

Conflict of interest

The authors declare no conflict of interest.

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PLoS one

Genetic Variants of Human Granzyme B Predict Transplant Outcomes after HLA Matched Unrelated Bone Marrow Transplantation for Myeloid Malignancies

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Abstract

Serine protease granzyme B plays important roles in infections, autoimmunity, transplant rejection, and antitumor immunity. A triple-mutated granzyme B variant that encodes three amino substitutions (Q48R, P88A, and Y245H) has been reported to have altered biological functions. In the polymorphism rs8192917 (2364A>G), the A and G alleles represent wild type QPY and RAH mutant variants, respectively. In this study, we analyzed the impact of granzyme B polymorphisms on transplant outcomes in recipients undergoing unrelated HLA-fully matched T-cell-replete bone marrow transplantation (BMT) through the Japan Donor Marrow Program. The granzyme B genotypes were retrospectively analyzed in a cohort of 613 pairs of recipients with hematological malignancies and their unrelated donors. In patients with myeloid malignancies consisting of acute myeloid leukemia and myelodysplastic syndrome, the donor G/G or A/G genotype was associated with improved overall survival (OS; adjusted hazard ratio [HR], 0.60; 95% confidence interval [CI], 0.41–0.89; $P = 0.01$) as well as transplant related mortality (TRM; adjusted HR, 0.48; 95% CI, 0.27–0.86, $P = 0.01$). The recipient G/G or A/G genotype was associated with a better OS (adjusted HR, 0.68; 95% CI, 0.47–0.99; $P = 0.05$) and a trend toward a reduced TRM (adjusted HR, 0.61; 95% CI, 0.35–1.06; $P = 0.08$). Granzyme B polymorphism did not have any effect on the transplant outcomes in patients with lymphoid malignancies consisting of acute lymphoid leukemia and malignant lymphoma. These data suggest that there is an association between the granzyme B genotype and better clinical outcomes in patients with myeloid malignancies after unrelated BMT.

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Introduction

Hematopoietic stem cell transplantation (HSCT) represents the only potentially curative option for many malignant conditions. Although substantial improvements in the supportive care of transplanted patients have been achieved in recent years, the profound compromise in the immune system associated with HSCT constitutes a significant risk for life threatening complications including GVHD, severe infections and disease relapse.[1] HLA matching represents the major genetic determinant in clinical outcomes after allogeneic HSCT, however, several studies have suggested that non-HLA genes associated with immune functions are also involved in determining the clinical outcome.[2] Single nucleotide polymorphisms (SNPs) in genes involved in the immune response to infections and inflammatory reactions have been identified as additional predictive markers of clinical outcomes in HSCT.[3,4,5,6,7,8,9,10,11,12,13,14]

Following HSCT, cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells, mainly derived from the donor, constitute the most important effector cells that eliminate allogeneic cells, including malignant cells, virus-infected cells and healthy cells. The destruction of the target cells occurs by at least one of the three defined mechanisms: TNF-α release, the Fas/Fas ligand interaction, and the granzyme/perforin pathway.[15] The later has been postulated as being the predominant mechanism for immune-mediated apoptosis of allogeneic cells.[16,17]

Granzyme B, the most abundant serine protease stored in secretory granules of CTLs and NK cells, is released upon target cell recognition, then specifically enters into the target cell cytoplasm via perforin, finally leading to target cell lysis.[15] Although the induction of target cell death by its pro-apoptotic properties has been considered the central function of granzyme B, growing evidence indicates that this protease also possesses additional non-death-related functions. These non-classical or extracellular functions are perforin-independent mechanisms and include immunosuppression, receptor cleavage, and cytokine-like effects.[15,18,19] Initially believed to be expressed exclusively by NK cells and CTLs, recent reports have shown that granzyme B can be expressed by various additional cell types, such as mast cells, neutrophils, dendritic cells (DCs), B cells, keratinocytes, chondrocytes, and vascular smooth muscle cells.[20,21,22,23,24]

Granzyme B is involved in the pathophysiology of viral and bacterial infections, solid organ rejection, autoimmune diseases, and antitumor immunity.[25,26,27,28,29,30] In the granzyme B gene, a triple-mutated allele (Q48R, P88A, and Y245H) in strong linkage disequilibrium is found in European, African, and Asian populations, including the Japanese population, at an allelic frequency of 25–30%.[31,32] The biological and functional relevance of the RAH mutant granzyme B, however, still remains controversial. Although it was reported that the RAH variant was incapable of inducing apoptosis,[31] and γδT cells derived from donors possessing the RAH variant had impaired cytotoxicity against target cells;[33] other studies have reported that RAH mutant granzyme B displays normal proteolytic and cytotoxic properties[34] and the cytotoxicic activity of T lymphocytes did not differ among donors with QPY or RAH genotypes.[32]

In this study, we hypothesized that a defect of inducing apoptosis in mutant granzyme B could influence the clinical outcomes of HSCT. To test this hypothesis, we investigated the influence of the QPY/RAH variants on the clinical outcomes after HSCT. Because these variants are in clear linkage disequilibrium, the study was focused on genotyping the polymorphism rs8192917 (2364A>G) in the granzyme B gene, which results in Q48R variants, and analyzed its impact on the clinical outcomes of patients undergoing allogeneic bone marrow transplantation (BMT) using an HLA allele-matched unrelated donor. The data herein show that the donor G/G or A/G allele, which represents mutant granzyme B, is associated with a significantly improved overall survival (OS) and reduced transplant-related mortality (TRM) in patients with myeloid malignancies.

Methods

Patients

Granzyme B genotyping was performed on 613 recipients with hematological malignancies and their unrelated donors who underwent BMT through the Japan Marrow Donor Program (JMDP) with T-cell-replete marrow from HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 allele-matched donors between January 1993 and December 2007. The HLA genotypes of patients and donors were determined by the Luminex microbead method, as described previously (Luminex 100 System; Luminex, Austin, TX).[35,36] Although the Luminex microbead method does not provide unambiguous HLA 4-digit typing for all genotypes, the JMDP has confirmed that this method can identify all HLA alleles with >0.1% frequency among the Japanese population.[37]

None of the present patients had a history of any prior transplantation. The final clinical survey of these patients was completed by November 1, 2008. The diagnoses were acute myeloid leukemia (AML) in 240 (39%), acute lymphoblastic leukemia (ALL) in 170 (28%), myelodysplastic syndrome (MDS) in 113 (18%), and malignant lymphoma (ML) in 90 (15%; **Tables 1 and 2**). The recipients were defined as having standard risk disease if they had AML or ALL in the first complete remission, ML in any complete remission, or MDS. All others were designated as having high-risk disease. The myeloid malignancies include AML and MDS, and the lymphoid malignancies included ALL and ML. Cyclosporine- or tacrolimus-based regimens were used in all patients for GVHD prophylaxis, and anti-T cell therapy, such as anti-thymocyte globulin and *ex vivo* T cell depletion were not in any of the patients. All patients and donors gave their written informed consent at the time of transplantation to participate in molecular studies of this nature according to the declaration of Helsinki. This project was approved by the Institutional Review Board of Kanazawa University Graduate School of Medicine and the JMDP.

Granzyme B genotyping

Genotyping of granzyme B was performed using the TaqMan-Allelic discrimination method in a StepOne Plus Real Time PCR system (Applied Biosystems, Foster City, CA, USA), and the results

Table 1. Donor and recipient characteristics (first part).

Variable	No.	Ratio
No. of cases	613	
Recipient age, years		
Median	36	
Range	1–70	
Donor age, years		
Median	34	
Range	20–57	
Year of transplant		
Median	2002	
Range	1993–2007	
Recipient Granzyme B genotype		
G/G	30	5%
A/G	202	33%
A/A	381	62%
Donor Granzyme B genotype		
G/G	27	4%
A/G	194	32%
A/A	392	64%
Recipient sex		
Male	383	62%
Female	230	38%
Donor sex		
Male	402	66%
Female	210	34%
Missing	1	0%
Donor/recipient sex		
Sex matched	409	67%
Female/male	92	15%
Male/female	111	18%
Missing	1	0%

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Table 2. Donor and recipient characteristics (second part).

Variable	No.	Ratio
Disease		
Acute myeloid leukemia	240	39%
Myelodysplastic syndrome	113	18%
Acute lymphoblastic leukemia	170	28%
Malignant lymphoma	90	15%
Disease stage		
Standard risk	357	58%
High risk	256	42%
ABO matching		
Major or/and minor mismatch	246	40%
Major mismatch	136	22%
Minor mismatch	126	21%
Bidirectional	18	3%
Missing	7	1%
Conditioning regimen		
Myeloablative	499	81%
Reduced intensity	114	19%
With total body irradiation	472	77%
Pretransplant CMV serostatus		
CMV positive recipient	440	72%
Missing	70	11%
GVHD prophylaxis		
With cyclosporine	296	48%
With tacrolimus	314	51%
Missing	3	0%
TNC, $\times 10^8$ per kg		
Median	4.9	
Range	0.1–79.1	

Abbreviations: TNC: total nucleated cell count harvested.
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were analyzed using the Allelic Discrimination software program (Applied Biosystems). The genotyping assay was conducted in 96-well PCR plates. The amplification reaction contained template DNA, TaqMan universal master mix and the specific probe designed for SNP rs8192917 (2364A>G) of granzyme B (product No. C_2815152_20 ; Applied Biosystems).

Data management and statistical analysis

The 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. The pre-transplant cytomegalovirus (CMV) serostatus was routinely tested for only patients, but not the donors. Engraftment was confirmed by an absolute neutrophil count of more than 0.5×10^9/L for at least 3 consecutive days. On collecting data, acute and chronic GVHD were diagnosed and graded using the previous criteria[38,39] and data using updated criteria for assessment of GVHD[40,41] were not available in our cohort. The OS was defined as the number of days from transplantation to death from any cause. Disease relapse was defined as the number of days from transplantation to disease relapse. TRM was defined as death without relapse. Any patients who were alive at the last-follow-up date were censored. The data about causative microbes of infections and postmortem changes in cause of death, as well as the data on supportive care, including prophylaxis for infections and therapy for GVHD, which were given on an institutional basis, were not available for this cohort.

The analysis was performed using the Excel 2007 (Microsoft Corp, Redmond, WA, USA) and modified R (The R Foundation for Statistical Computing, Perugia, Italy) software programs.[42,43] The probability of overall survival (OS) was calculated using the Kaplan-Meier method and compared using the log-rank test. The probabilities of TRM, disease relapse, acute GVHD, chronic GVHD, and engraftment were compared using the Grey test[44] and analyzed using the cumulative incidence analysis,[42] considering relapse, death without disease relapse, death without acute GVHD, death without chronic GVHD, and death without engraftment as respective competing risks. The variables were recipient age at time of transplantation, sex, pretransplant CMV serostatus, disease characteristics (disease type, disease lineage and disease risk at transplantation), donor characteristics (age, sex, sex compatibility, and ABO compatibility), transplant characteristics (conventional or reduced-intensity conditioning,[45] total body irradiation-containing regimen, tacrolimus versus cyclosporine, and total nucleated cell count harvested per recipient weight [TNC]), and the year of transplantation. The median was used as the cutoff point for continuous variables. The chi-square test and Mann-Whitney U test were used to compare two groups. The Hardy-Weinberg equilibrium for the granzyme B gene polymorphism was tested using the Haploview software program.[6]

Multivariate Cox models were used to evaluate the hazard ratio associated with the granzyme B polymorphism. Covariates found to be $P\leq0.10$ in the univariate analyses were used to adjust the hazard ratio. The covariates were selected according to myeloid and lympoid malignancies.

For both the univariate and multivariate analyses, P values were two sided, and outcomes were considered to be significant for $P\leq0.05$.

Results

The frequencies of the granzyme B genotypes

Granzyme B gene polymorphism was analyzed in 613 unrelated bone marrow donor-transplant recipient pairs (**Tables 1 and 2**). The genotype frequencies of G/G, A/G and A/A were 5%, 33% and 62% in recipients and 4%, 32% and 64% in donors. These were similar to HapMap data in the Japanese (9%, 29% and 62%, respectively) and European (5%, 35% and 60%, respectively) populations, and thus were in accord with the Hardy-Weinberg equilibrium ($P=0.79$).

Transplant outcome according to the granzyme B genotype

The median follow-up duration in the cohort was 55 months among the survivors (range 4 to 168 months), and 191 recipients (31%) had relapsed or progressed, and 309 (50%) had died. Eighteen patients (3%) died before engraftment. The donor and recipient granzyme B genotype did not significantly influence the cumulative incidence of engraftment (data not shown).

The transplant outcomes according to the granzyme B genotype are summarized in **Table 3**. Patients with myeloid malignancies, which included AML and MDS, who received transplants from donors with the G/G or A/G genotype had a significantly better 5-year OS (58% vs. 42%, $P=0.01$; **Fig. 1A**) and a trend toward lower 5-year relapse rate (27% vs. 36%, $P=0.09$) than those receiving transplants from donors with the A/A genotype. No

Table 3. The results of the univariate analysis of the association of the Granzyme B genotype with clinical outcomes after transplantation.

Variable	No.	5-year OS	*P*	5-year TRM	*P*	5-year relapse	*P*	II-IV acute GVHD	*P*	Chronic GVHD	*P*
Myeloid malignancy	353										
Recipient Granzyme B genotype											
A/G or G/G	139	52%	0.13	21%	0.17	33%	0.71	25%	0.23	40%	0.14
A/A	214	46%		26%		33%		31%		49%	
Donor Granzyme B genotype											
A/G or G/G	141	58%	0.01	20%	0.21	27%	0.10	28%	0.72	47%	0.79
A/A	212	42%		26%		37%		30%		45%	
Lymphoid malignancy	260										
Recipient Granzyme B genotype											
A/G or G/G	93	48%	0.14	24%	0.26	35%	0.97	35%	0.66	31%	0.44
A/A	167	43%		26%		34%		33%		36%	
Donor Granzyme B genotype											
A/G or G/G	80	43%	0.93	29%	0.60	33%	0.78	34%	0.88	32%	0.49
A/A	180	46%		24%		35%		34%		36%	

Abbreviations: OS, overall survival; TRM, Transplant-related mortality.
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difference was noted in the TRM, II-IV acute GVHD, or chronic GVHD in relation to the donors' polymorphism status. A comparison between the donor G/G and A/G genotypes showed no significant difference in OS (71% vs. 56%, $P = 0.36$), TRM (6% vs. 23%, $P = 0.36$), or the relapse rate (30% vs. 26%, $P = 0.65$). When patients with AML and MDS were separately analyzed, the donor G/G or A/G genotypes remained statistically significant for a better OS in AML patients (58% vs. 45%; **Fig. 1B**), and had a tendency to be related to a better OS in MDS patients (58% vs. 37%; **Fig. 1C**). In patients with lymphoid malignancies consisting of ALL and ML, the donor granzyme B genotype had no significant effects on transplant outcomes (**Table 3**). This was true even when ALL and ML were separately analyzed (data not shown).

Multivariate analysis

All of the factors found to be significant in the univariate analyses were included in the model. In patients with myeloid malignancies, the G/G or A/G genotype in donors were statistically significant in the multivariate analyses for better OS (adjusted hazard ratio [HR], 0.60; 95% confidence interval [CI], 0.41–0.89; $P = 0.01$; **Table 4**) and TRM (adjusted HR, 0.45; 95% CI, 0.25–0.80; $P = 0.01$) when adjusted for the other factors in the models. Despite not evident in the univariate analysis, the multivariate analysis revealed the donor granzyme B G/G or AG genotype was associated with lower incidence of chronic GVHD (adjusted HR, 0.61; 95% CI, 0.37–0.99; $P = 0.05$; **Table 5**). In the independent analyses for AML patients and MDS patients, beneficial effects on OS by the donor G/G or A/G genotype were also found, which was close to being significant in both the AML patients (adjusted HR, 0.68; 95% CI, 0.42–1.09; $P = 0.10$) and the MDS patients (adjusted HR, 0.61; 95% CI, 0.35–1.08; $P = 0.09$). In addition, the recipient G/G or A/G genotype was associated with a significantly better OS (adjusted HR, 0.68; 95% CI, 0.47–0.99; $P = 0.05$) and a trend toward a reduced TRM (adjusted HR, 0.61; 95% CI, 0.35–1.06; $P = 0.08$). The difference between the donor G/G and A/G genotype did not reach statistical significance in relation to transplant outcomes (data not shown). The granzyme B genotype did not significantly influence the transplant outcomes in patients with lymphoid malignancies.

Discussion

The current study showed that the granzyme B G/G or A/G genotype at rs8192917 (2364A>G) in the donor side representing the triple variant RAH granzyme B was associated with a significantly better OS and TRM compared to the granzyme A/A genotype, corresponding to wild type QPY granzyme B, for patients with myeloid malignancies receiving HLA-matched unrelated BMT through the JMDP. The G/G or A/G genotypes in the recipient also significantly improved the OS, as well the TRM, although to a lesser extent. This is the first report to show that the granzyme B polymorphism affects transplant outcomes.

The beneficial effects of the G/G or A/G genotype were absent in patients with lymphoid malignancies, irrespective of whether it was ALL or ML. A possible explanation for this may be that ALL and ML cells express the apoptosis inhibitor Bcl-2[46] and the endogenous inhibitor of granzyme B, proteinase inhibitor 9 (PI-9).[47,48,49,50] The expression of these two factors by malignant lymphoid cells may protect them from granzyme B-induced apoptosis and proteolysis[46,48] and might thus negate the differential effects of the different granzyme B genotypes.

Based on the traditional view that the triple-mutated granzyme B has an impaired pro-apoptotic function, it was expected that the presence of the RAH variant would predict an adverse clinical outcomes after HSCT, namely poor survival or an increased relapse rate. The results presented here, however, do not support that assumption. The mechanisms by which the mutant granzyme B genotype improved transplant outcomes remain unclear. This may be due, in part, because the reports on the biochemical and physiological properties of the triple variant RAH granzyme B are still controversial.

Although an initial study[31] reported that RAH granzyme B was unable to induce apoptosis in tumor cell lines, it was later

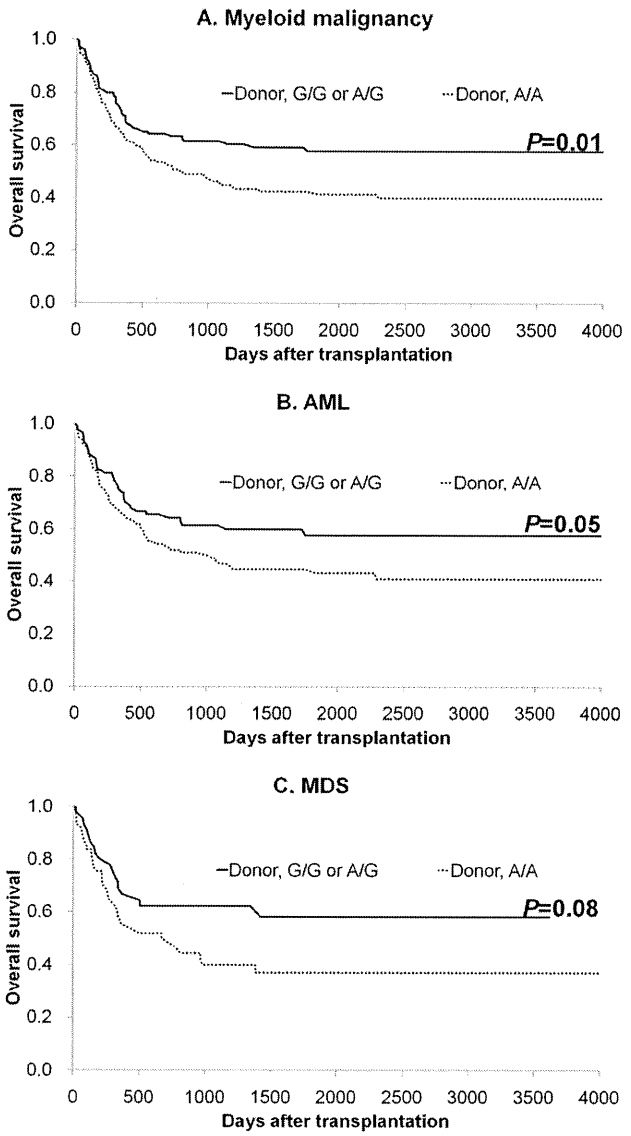

Figure 1. The Kaplan-Meier analysis of OS after BMT according to the donor granzyme B genotype in patients with myeloid malignancies (A), AML (B), and MDS (C).
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reported by others that RAH granzyme B retains its pro-apoptotic activity.[34] In addition to the classical role of granzyme B in mediating apoptosis within target cells by NK cells and CTLs, increasing evidence shows that extracellular granzyme B also has alternative functions, including extracellular matrix remodeling, immunosuppressive and cytokine-like effects.[18,19,24,51,52] A recent report[19] showed that human DCs abundantly secrete granzyme B, which can suppress T-cell expansion. Another report revealed a pivotal function of granzyme B in immunosuppression directed by regulatory T cells, leading to promotion of tumor escape.[52] In addition, extracellular granzyme B potentially induces apoptosis in various organs and tissues, thus leading to chronic inflammatory, autoimmune, and degenerative diseases.[18,24] In line with these observations, it is plausible that in patients receiving HSCT, extracellular granzyme B could contribute to significant effects, such as modulation of T-cell functions and organ damage, because high serum levels of extracellular granzyme B have been reported in HSCT recipients.[29]

Based on the results presented herein, it may therefore be reasonable to hypothesize that the granzyme B variants have differential biochemical properties whose biological consequences are more relevant on the non-classical functions exerted by the extracellular granzyme B. The analysis of patient serum may offer useful information on this issue, although these samples were not available for the present study. The fact that functional granzyme B is also secreted by nonhematopoietic cells, including keratinocytes, chondrocytes, and smooth muscle cells[18,53] may explain the findings that granzyme B variants in the recipient side, in addition to that in the donor side, had an impact on the transplant outcomes. Furthermore, this finding supports the view that the presence of the triple-mutated Granzyme B is indeed responsible for the beneficial effect in HSCT for myeloid malignancies.

The effects of the granzyme B G/G or A/G genotype on the reduced TRM in patients with myeloid malignancies might be a consequence of increased resistance to infections in these recipients. This hypothesis, although attractive, is highly speculative and is not supported by the present study because of the unavailability of data on the causes of infections in this cohort. Further studies will be needed to clarify whether the granzyme B genotypes can differentially affect the responses of patients against infections.

Two recent reports have described a significant correlation between disease susceptibility and the RAH/QPY polymorphism in the granzyme B gene. The wild type QPY genotype was associated with an increased incidence of Epstein-Barr-virus-associated

Table 4. The results of a multivariate analysis of the association of the Granzyme B genotype with the clinical outcomes after transplantation.

Variable	OS			TRM			Relapse		
	Adjusted HR	95% CI	*P*	Adjusted HR	95% CI	*P*	Adjusted HR	95% CI	*P*
Myeloid malignancy									
Recipient Granzyme B genotype, G/G or A/G	0.68	0.47–0.99	0.05	0.61	0.35–1.06	0.08	0.99	0.65–1.51	0.97
Donor Granzyme B genotype, G/G or A/G	0.60	0.41–0.89	0.01	0.45	0.25–0.80	0.01	0.75	0.48–1.15	0.19
Lymphoid malignancy									
Recipient Granzyme B genotype, G/G or A/G	0.99	0.60–1.57	0.96	0.93	0.44–1.96	0.84	1.40	0.84–2.34	0.20
Donor Granzyme B genotype, G/G or A/G	0.72	0.43–1.28	0.23	0.84	0.32–2.22	0.72	0.87	0.49–1.56	0.65

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Table 5. The results of a multivariate analysis of the association of the Granzyme B genotype with GVHD after transplantation.

	II-IV acute GVHD			Chronic GVHD		
Variable	Adjusted HR	95% CI	*P*	Adjusted HR	95% CI	*P*
Myeloid malignancy						
Recipient Granzyme B genotype, G/G or A/G	0.78	0.51–1.19	0.24	0.83	0.53–1.31	0.42
Donor Granzyme B genotype, G/G or A/G	0.94	0.62–1.43	0.76	0.61	0.37–0.99	0.05
Lymphoid malignancy						
Recipient Granzyme B genotype, G/G or A/G	0.90	0.55–1.45	0.69	0.90	0.54–1.50	0.69
Donor Granzyme B genotype, G/G or A/G	1.07	0.65–1.76	0.79	1.13	0.68–1.89	0.64

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hemophagocytic lymphohistiocytosis (HLH) in children.[32] Conversely, a subsequent study reported an association of the mutant RAH genotype with the incidence of breast cancer.[33] However, to link the genetic susceptibility of granzyme B to disease based on the presented data is difficult, because no patient developed HLH or breast cancer following HSCT in the current cohort.

In conclusion, the present data suggest that the granzyme B polymorphism may affect the prognosis after BMT from an unrelated donor, and therefore, the granzyme B genotyping in transplant donors and recipients may provide opportunities to choose an ideal donor. However, care should be made in drawing conclusions, because the number of patients evaluated in the present study is limited. Experimental evidence is also required to substantiate the effects of extracellular granzyme B according to the polymorphism on organ and tissue damage. Further studies are warranted to ascertain whether the findings of this study can be extended to other disease groups, other stem cell sources, or HLA-mismatched transplantation, as well as to validate the present data.

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Author Contributions

Conceived and designed the experiments: LJE AT. Performed the experiments: LJE KY. Analyzed the data: AT KN. Contributed reagents/materials/analysis tools: AT MO TK HS HA KM SO MI TF YM YK. Wrote the paper: AT LJE. Conducted the study: SN.

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CASE REPORT
Malignant Diseases

Acute Respiratory Distress Syndrome as an Initial Presentation of Hemophagocytic Lymphohistiocytosis After Induction Therapy for Acute Myeloid Leukemia

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A 7-month-old girl with acute myeloid leukemia (AML) developed acute respiratory distress syndrome (ARDS) during the pancytopenic period after induction chemotherapy. Respiratory failure did not improve despite intensive treatments. Eventually, hemophagocytic lymphohistiocytosis (HLH) was diagnosed based on hemophagocytosis in bone marrow, and high soluble interleukin-2 receptor (sIL-2R) and ferritin levels. Even after cyclosporin A was started against HLH, she did not recover. Autopsy showed macrophage proliferation in bone marrow and lymph nodes. HLH should be considered, even in the pancytopenic period after chemotherapy, when patients develop ARDS that does not respond to supportive therapies.

Keywords acute respiratory distress syndrome, ferritin, hemophagocytic lymphohistiocytosis, hypercytokinemia, sIL-2R

Hemophagocytic lymphohistiocytosis (HLH) is characterized by multisystem inflammation, a reactive process resulting from the prolonged and intense activation of antigen-presenting cells and CD8 + T cells, and the excessive proliferation and ectopic migration of T cells [1]. Abnormalities in the function of natural killer (NK) cells have also been identified as characteristics of HLH [1]. HLH is caused by hypercytokinemia that results from the failure of natural immune down-regulation due to defective NK and cytotoxic T-lymphocyte function [1]. Clinical symptoms include fever, splenomegaly, pancytopenia, and lymphadenopathy. HLH can be considered to be either primary or secondary. Primary HLH, also called familial HLH, is the autosomal recessive form and usually occurs in infants [1, 2]. Secondary HLH is associated

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with a variety of underlying diseases, including infections, malignancies, and autoimmune disorders. HLH associated with malignancy is usually seen at the initial presentation of malignancy, which is typically lymphoid malignancy, including T-cell and NK-cell tumors [1]. This can sometimes even mask the malignancy itself [1]. Recently, chemotherapy-related HLH has been recognized as a rare lethal adverse event that is a consequence of treatment-related immunosuppression [3]. Whatever the cause, the early diagnosis of HLH is very crucial for an appropriate treatment. We describe here a 7-month-old infant who developed acute respiratory distress syndrome (ARDS) as a manifestation of HLH after induction chemotherapy for acute myeloid leukemia (AML).

CASE REPORT

A girl was delivered from healthy parents after an uncomplicated pregnancy by spontaneous vaginal delivery after 38 weeks, and weighed 3398 g. Her development was normal, as had been that of her elder brother. At 7 months, she was referred to our hospital because of persistent vomiting, purpura in the lower extremities, and leukocytosis. On admission, she appeared remarkably ill with an anemic face and purpura in the lower extremities. She had bilateral cervical, axillary, and inguinal lymphadenopathy. Liver and spleen were enlarged to 6 and 3 cm below the costal margin, respectively. Initial laboratory findings revealed leukocytosis (white blood cell [WBC] count: 152,130/μL with 54.5% blasts), anemia, and thrombocytopenia. The diagnosis of AML was established based on a bone marrow (BM) smear that showed 76.6% monoblasts (M5a type in the French-American-British [FAB] classification). Leukemic blasts were positive for CD 11b, CD 15, CD 33, CD 38, CD 56, CD 64, and cytoplasmic myeloperoxidase (MPO) by flow cytometry. Chromosomes of BM cells revealed 46, XX. She received remission induction chemotherapy that included etoposide at 150 mg/m^2 for 5 days, mitoxantrone at 5 mg/m^2 for 5 days, cytosine-arabinoside 2000 mg/m^2 for 7 days, intrathecal methotrexate 3 mg, cytosine-arabinoside 6 mg, and hydrocortisone 10 mg on day 6. On day 8, leukemic blasts disappeared from the peripheral blood. Her hepatosplenomegaly and lymphadenopathy receded at around day 10. She received additional intrathecal methotrexate, cytosine-arabinoside, and hydrocortisone on day 13, since the initial cerebrospinal fluid (CSF) was positive for leukemic cells (cell count: 11/μL).

She developed febrile neutropenia on day 11. Parenteral antibiotic therapy was started. Although blood cultures were negative, the high fever persisted. Finally, on day 14, she developed a shock-like state that required the administration of catecholamine. Granulocyte colony-stimulating factor (G-CSF) was started for persistent neutropenia. The clinical course was complicated with ileus. Abdominal distension continued, along with bile-like drainage from the gastric tube; this was aggravated by jaundice with predominantly direct-bilirubin. She also exhibited disseminated intravascular coagulation (DIC) after gastrointestinal bleeding. However, these conditions were stabilized by blood transfusion and treatment for DIC. No new bleeding was noted from day 32, and fever subsided on day 33 with recovery of the neutrophil count to 540/μL. Her symptoms of jaundice and hyperbilirubinemia were also improving.

On day 34 she developed sudden dyspnea with 70% oxygen saturation in room air and pyrexia. A chest x-ray showed a ground-glass appearance in the bilateral lungs. Arterial blood gas analysis showed impaired pulmonary oxygenation (Pao_2/Fio_2 118). She was placed on mechanical ventilation for respiratory failure. No pathogenic bacterial, fungal or viral agents, including cytomegalovirus (CMV), human herpes virus 6 (HHV6), adenovirus (ADV), and Epstein-Barr virus (EBV), were identified in microbiological cultures, polymerase chain reaction (PCR), and serological