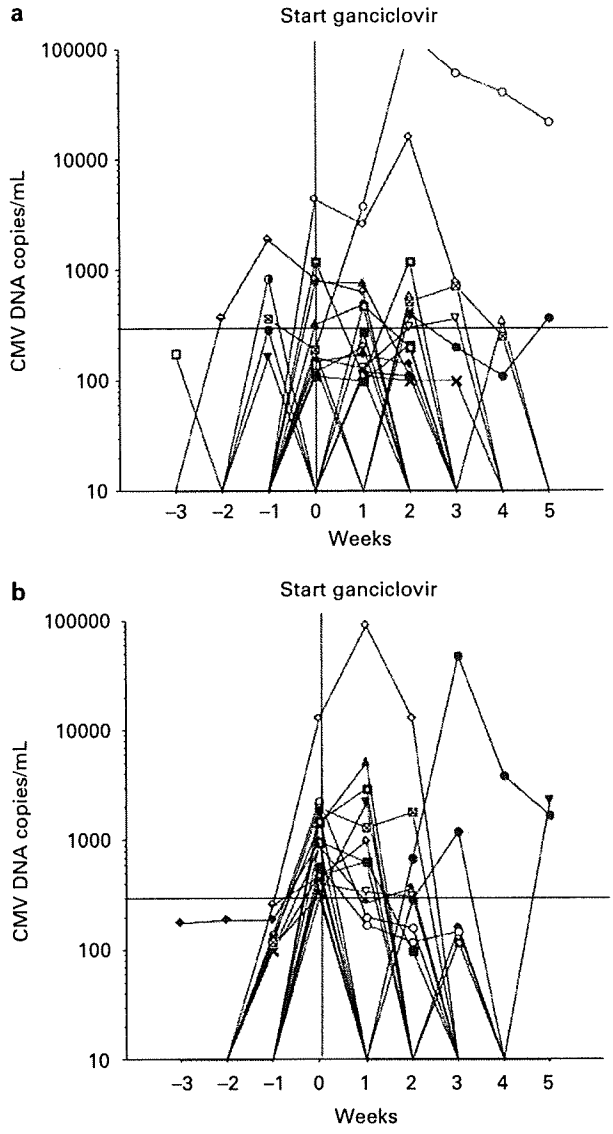

Figure 5 Serial changes in CMV DNA copy number in patients who received preemptive therapy in the antigenemia group (**a**) and in the PCR group (**b**). Week 0 represents the day ganciclovir was started.

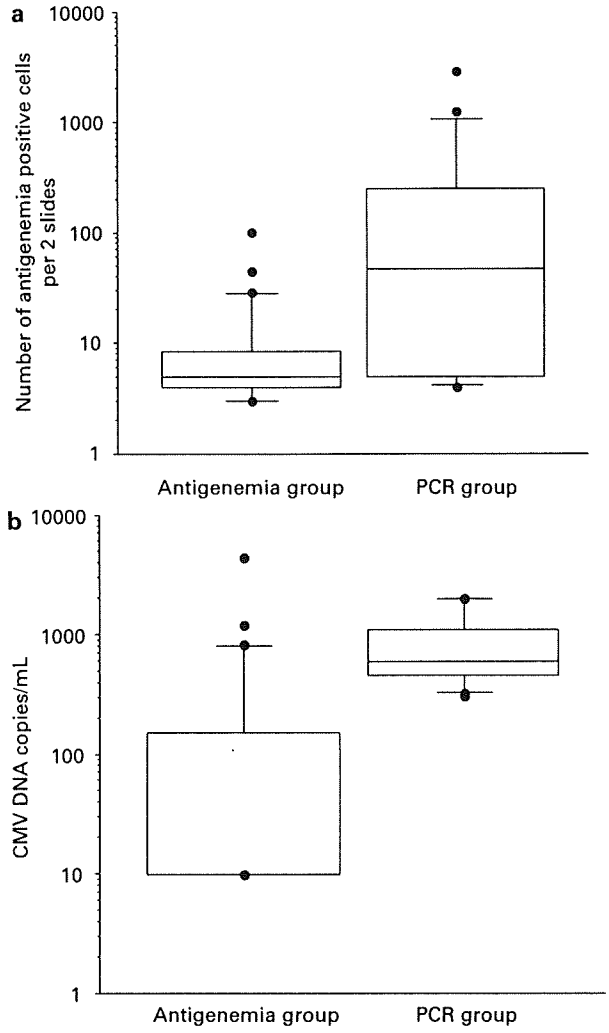

Figure 6 The number of antigenemia-positive cells (**a**) and the CMV DNA copy number at the start of preemptive therapy (**b**), grouped according to the randomization arm. The box-and-whisker plot shows 10, 25, 50, 75 and 90 percentile values. Outliers are indicated by dots.

interval for the difference in the successful prevention rate was just outside the predefined lower limit of −10%, and therefore, we could not show the noninferiority of the PCR group, the incidence of CMV disease was limited to two patients even in the PCR group. In addition, prevention of CMV pneumonia, the main aim of preemptive therapy, was completely achieved in both groups. These findings suggest that an antigenemia assay with a cutoff of three positive cells per two slides was too sensitive and resulted in the unnecessary use of GCV.

The unnecessary use of GCV may be reduced if the cutoff value for the antigenemia assay is increased. The antigenemia assay has already been shown to be not sensitive enough for detecting gastrointestinal involvement by CMV even with a low threshold.[21] In this study, the median number of antigenemia-positive cells at the start of GCV treatment was 47 in the 19 patients who received preemptive therapy in the PCR group. Figure 7 shows the serial changes in the number of antigenemia-positive cells in the patients of the PCR group who developed positive antigenemia that reached the threshold, but who did not receive GCV at that time. In about half of the patients, antigenemia spontaneously became negative without GCV treatment. On the other hand, seven patients developed high-grade antigenemia of over 100 positive cells per two slides. However, GCV was started when the number of positive cells was 260 (median, range: 73–1262 cells) and none of these patients developed CMV disease. Although patients who developed grade II–IV acute GVHD or who received steroid at 0.5 mg/kg or higher experienced high-grade antigenemia more frequently than those who did not

develop grade II–IV acute GVHD and did not receive steroid (Figures 7a and b), the use of GCV was comparable (54.5 vs 40%, $P=0.67$). Thus, although it is difficult to determine the appropriate cutoff value for the antigenemia assay, we thought that it may be worth trying to apply a cutoff value of 20 positive cells per two slides, which we are already safely using in allogeneic hematopoietic SCT from an HLA-matched sibling donor,[20] to transplantation from an unrelated donor.

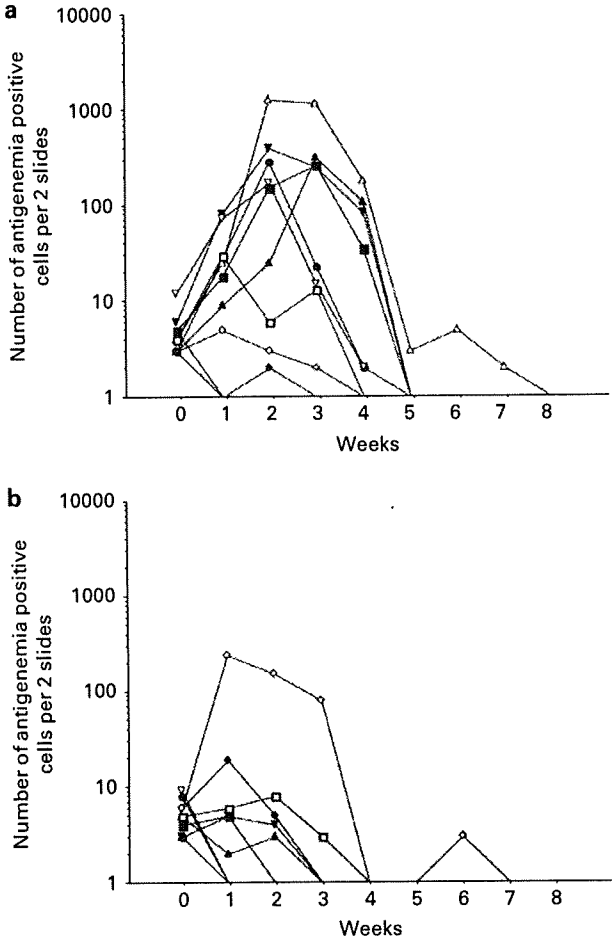

Figure 7 Serial changes in the number of antigenemia-positive cells in the PCR group patients who developed positive antigenemia that reached the threshold, but who did not receive ganciclovir. (a) Patients who developed grade II–IV acute GVHD or who received steroid at 0.5 mg/kg or more. (b) Patients who did not develop grade II–IV acute GVHD and did not receive steroid.

Although Boeckh *et al.*[3] reported a 14% incidence of early CMV disease using the same cutoff as in the current study, the incidences of positive antigenemia at any level and three or more positive cells per two slides were similar to those in this study (79 and 70% in Boeckh's study and 89 and 73% in the current study). Therefore, the higher incidence of early CMV disease probably resulted from the high incidence (35%) of grade III–IV acute GVHD in their study rather than from the difference in the method used for the antigenemia assay, as acute GVHD is one of the strongest risk factors for CMV disease.

Nevertheless, it is important to note that the sensitivity and specificity of these assays vary depending on the methodology used.[9,22–24] In fact, the unexpected differences in the sensitivities of the two assays in this study could be explained by the difference in the methodology used in the antigenemia assay. The cutoffs used for the antigenemia assay and real-time PCR were determined based on our previous study in which HRP-C7 Ab was used in the antigenemia assay.[18] In this study, however, we used C10/C11 Ab in the antigenemia assay, as this Ab has been used worldwide. Although we did not believe that there are clinical differences between these two antigenemia assays,[6,7,20] we should have tested the correlation between the results of plasma PCR and the antigenemia assay using C10/C11 Ab. Fortunately, the unexpected difference in the sensitivity in these assays contributed to the finding that the antigenemia assay with the current cutoff was too sensitive as a trigger for deciding when to start preemptive therapy. These data are valid only when the same methodology is used, and standardization of the methods is warranted.[25,26]

In conclusion, CMV colitis could not be completely prevented by the current preemptive strategy using the peripheral blood samples, but CMV pneumonia was completely prevented in both groups. The initiation of GCV at 5 mg/kg/day was confirmed to be safe, provided the CMV load continues to be monitored. Plasma PCR with a cutoff at 300 copies per ml seemed to be appropriate for monitoring CMV reactivation after transplantation. The cutoff number of positive cells should be raised above that used here when using an antigenemia assay. However, the appropriateness of the threshold of these assays may be different on the basis of the methodology and patient background, such as the risk of GVHD, and therefore, it is difficult to generalize.

Table 3 CMV load in patients who developed CMV disease

Age/sex	*Acute GVHD*	*Onset/affected organ of CMV disease*		*−3 weeks*	*−2 weeks*	*−1 week*	*Onset*
UPN32 38/M (PCR group)	Grade II	Day 56/colitis	PCR	(−)	260	13 000[a]	93 000
			Ag	(−)	(−)	2921	5467
UPN35 36/M (PCR group)	Grade II	Day 46/colitis	PCR	(−)	(−)	(−)	(−)
			Ag	0	0	2	12
UPN70 38/M (Antigenemia group)	Grade II	Day 50/colitis	PCR	(−)	(−)	110	100
			Ag	2	(−)	5[a]	99

[a]Preemptive therapy was started.

Conflict of interest

The authors declare no conflict of interest.

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Original article

The effect of different ATG preparations on immune recovery after allogeneic hematopoietic stem cell transplantation for sever aplastic anemia.

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Running head: Different ATG preparations on immune recovery

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Abstract

Anti-thymocyte globulin (ATG) is widely used in the conditioning regimen before allogeneic stem cell transplantation for aplastic anemia. However, there are several different preparations of ATG and little is known about the difference of their effects on transplantation outcome. Therefore, in this study, we retrospectively compared the effect of two different rabbit ATG preparations (Thymoglobulin; ATG-G and ATG-Fresenius; ATG-F) on immune recovery and cytomegalovirus infection after transplantation. The conditioning regimen was a combination of fludarabine, cyclophosphamide, and ATG. Low dose total body irradiation was added in alternative donor transplantation. Four patients received ATG-F at 5 mg/kg/day from day -7 to day -3, whereas ATG-G was given at 2.5 mg/kg/day from day -5 to day -2 in 3 patients. There was no graft rejection and no grade II-IV acute GVHD. All 3 patients in the ATG-G group developed positive cytomegalovirus antigenemia including two with high-grade antigenemia, whereas 2 of the 4 patients in the ATG-F group were persistently negative. Immunological evaluation on day 60 revealed that both CD4+ and CD8+ T-cell recoveries were delayed in the ATG-G group. These findings suggested that ATG-G has

a stronger immunosuppressive activity than the ATG-F with a dose ratio of 1 : 2.5.

Key words:

Hematopoietic stem cell transplantation

Anti-thymocyte globulin

Immune recovery

Cytomegalovirus

Graft-versus-host disease

Introduction

Allogeneic hematopoietic stem cell transplantation is a curative treatment for severe aplastic anemia. Graft rejection and graft-versus-host disease (GVHD) had been the major problems after stem cell transplantation. To decrease the incidence of these complications, anti-thymocyte globulin (ATG) was added to the standard conditioning regimen with high-dose cyclophosphamide at 200 mg/kg[1]. The incidences of graft rejection, grade II-IV acute GVHD, and chronic GVHD were suppressed to 4%, 29%, and 32%, respectively, after allogeneic transplantation from an HLA-matched family donor using a conditioning regimen of high-dose cyclophosphamide and horse ATG (ATGAM; Upjohn, Kalamazoo, MI) at 30 mg/kg/day from day -5 to day -3[1]. Although a recent randomized controlled trial did not show a statistically significant benefit of ATG[2], the sample size was not enough to detect a survival benefit of less than 20% and in fact, 5-year survival was slightly better in the cyclophosphamide-ATG group (80% vs. 74%).

However, we should be cautious that there were several different preparations of ATG. For example, immunosuppressive therapy using horse ATG (Lymphoglobulin, Institute Pasteur Merieux, Lyon, France) showed

better response rate than that using rabbit ATG (Zetbulin, ATG-F, Fresenius, Munich, Germany) as a treatment for severe aplastic anemia[3]. Nevertheless, there has been little information about the difference of the effect of ATG among different brands when used in a conditioning regimen. Therefore, in this study, we retrospectively compared the effect of two different ATG preparations on immune recovery and cytomegalovirus infection after transplantation.

Patients and Methods

We retrospective reviewed the records of patients who underwent allogeneic hematopoietic stem cell transplantation for severe aplastic anemia in this hospital between July 2007 and March 2009. The backbone of the conditioning regimen was a combination of fludarabine at 30 mg/m^2/day from day -6 to day -3 and cyclophosphamide at 25 mg/kg/day from day -6 to day -3[4]. Total body irradiation (TBI) at 2 Gy was added on day -1 for patients who received graft from an alternative donor (a donor other than HLA-matched relatives)[5]. Until November 2008, ATG-F at 5 mg/kg/day was administered from day -7 to day -3, whereas another rabbit ATG, thymoglobulin (ATG-G, previously Sangstat, currently Genzyme, Cambridge, USA), was given at 2.5 mg/kg/day from day -5 to day -2, after ATG-G became available in Japan in December 2008. Bone marrow was the preferred stem cell source, but peripheral blood stem cell graft was chosen to accelerate neutrophil recovery in 3 patients who had very severe aplastic anemia associated with active infection.

Prophylaxis against GVHD was performed with cyclosporine started at 3 mg/kg/day from day -1 as a continuous infusion in combination with

methotrexate at 10 mg/m^2 on day 1 and 7 mg/m^2 on days 3 and 6, and optionally on day 11 for alternative donor transplantation. The dose of cyclosporine was adjusted to maintain the blood level at 500 ng/mL[6]. Cyclosporine was changed to an oral form when it could be tolerated by the patient. Prophylaxis against bacterial, fungal and pneumocystis jiroveci infection consisted of levofloxacin, fluconazole, and sulfamethoxazole/trimethoprim. Acyclovir was given at 1,000 mg/day from days –7 to 35, followed by long-term low-dose (200 mg/day) administration[7, 8]. Granulocyte-colony stimulating factor (filgrastim) at 300 μg/day was administered until neutrophil recovery. Preemptive therapy against cytomegalovirus was performed by monitoring antigenemia using C10/C11 antibody[9, 10]. Host/donor T-cell chimerism was analyzed by sex-chromosome fluorescent *in situ* hybridization or the short tandem repeat method after transplantation[11].

Results

Patients

Seven patients underwent allogeneic stem cell transplantation between July 2007 and March 2009. The patient characteristics are summarized in Table 1. Four received ATG-F and three received ATG-G. Two patients with very severe aplastic anemia in the ATG-F group performed immediate stem cell transplantation, whereas the other 5 patients had received immunosuppressive therapy but did not respond well. The donor was matched sibling donor in 3, whereas 4 received graft from an alternative donor.

All patients achieved stable donor cell engraftment and complete donor-type chimerism within one month after transplantation. Grade I acute GVHD was observed in 2 patients in the ATG-G group, but treatment was not required. Two patients in each group developed chronic GVHD, including one each with extensive form. One patient in the ATG-G group (ATG-G-2) died of adenovirus viremia and fulminant hepatitis on day 109. The other 6 patients are alive and well with a median follow-up duration of 15 months (range 4.5 - 18.5), although 1 patient in the ATG-G group (ATG-G-3) still

requires blood transfusion on day 136 after transplantation.

Cytomegalovirus antigenemia

Cytomegalovirus antigenemia assay was performed at least once a week after engraftment. Figure 1 shows the serial changes in the number of antigenemia positive cells per 2 slides. All three patients in the ATG-G group developed positive antigenemia, whereas 2 of the 4 patients in the ATG-F group were persistently negative for CMV antigenemia. In addition, 2 patients developed high-grade antigenemia of more than 100 positive cells per 2 slides despite the preemptive administration of ganciclovir. On the other hand, 2 patients with positive antigenemia in the ATG-F group promptly responded to ganciclovir. None of the patients developed CMV disease.

Immune recovery

Immunophenotypic analyses were performed with flow cytometry, using a whole-blood lysis method on days 60. As shown in Figure 2, the number of CD3+CD8+ T cells was lower than 50 /μL in all 3 patients in the ATG-G group, whereas it ranged between 80 /μL and 1,249 /μL in the ATG-F group. Also, the number of CD3+CD4+ T cells was lower than 50 /μL in all 3

patients in the ATG-G group, whereas it was higher than 90 /μL in 3 of the 4 patients in the ATG-F group. On the other hand, the numbers of CD3-CD19+ B cells and CD3-CD56+ NK cells were equivalent between the two groups.

Discussion

ATG is a strong immunosuppressive agent and widely used not only in hematopoietic stem cell transplantation but also in solid organ transplantation. On the other hand, the risk of infectious complication may increase by the use of ATG. The benefit and risk may differ among different preparations of ATG. Basara *et al.* retrospectively compared the effect of ATG-G at 5 - 15 mg/kg in total and ATG-F at 45 - 60 mg/kg in total in hematopoietic stem cell transplantation for acute myeloblastic leukemia, and found that the incidence of chronic GVHD was significantly lower after ATG-F, although the incidence of acute GVHD was not different[12]. However, immune recovery or the incidence of infectious complications was not evaluated in the report. On the other hand, several randomized controlled trial have been performed to compare different ATG preparations to prevent acute rejection after renal transplantation. Two of these studies showed that ATG-G at 1.5mg/kg/day was superior to ATGAM at 15 mg/kg/day for the prevention of acute rejection[13, 14]. The other two studies showed that there was no significant difference between ATG-F and ATG-G [15, 16]. However, the sample sizes of these studies were too small to draw definite conclusions. A

large retrospective study from the Saint-Jacques University Hospital revealed that the incidences of CMV disease and posttransplant malignancies were significantly lower after ATG-F at 21 mg/kg in total than ATG-G at 6 - 13 mg/kg in total, although there was a tendency toward lower incidence of graft loss in the ATG-G group[17]. Therefore, ATG-G may have a stronger immunosuppressive activity than the ATG-F at 2 to 3 times higher doses.

In this study, ATG-F at 25 mg/kg in total was compared with ATG-G at 10 mg/kg in total. Immunological evaluation 60 days after transplantation revealed that the recoveries of CD4+ and CD8+ T cells were delayed in the ATG-G group. In addition, all patients in the ATG-G group developed positive CMV antigenemia and 2 of them showed poor response to ganciclovir. Although we could not deny the possibility that it was in part affected by the CMV-seronegativity of the donor, the antigenemia response to preemptive therapy has been shown to be strongly affected by the background immunosuppression status[18-20], and thus, this may also be an evidence for the strong immunosuppressive property of ATG-G.

The shortcoming of this study was the small number of patients.

However, it seemed unethical to increase the number of patients in the ATG-G group with the current dose of ATG-G, since too strong immunosuppression has resulted in frequent posttransplant complications. Another concern was the heterogeneity of the patient characteristics in terms of previous immunosuppressive treatments, donor type, stem cell source, and so on. Especially, the previous treatment with ATG might have affected the immune recovery after transplantation. However, the numbers of CD4+ and CD8+ T-cells just before the conditioning regimen were not different between the ATG-F and ATG-G groups (60 /μL vs. 94 /μL and 188 /μL vs. 134 /μL, respectively, in median). Nevertheless, we could not deny the possibility the timing of the ATG administration might have affected T-cell recovery after transplantation. ATG-G was infused one day closer to the day of transplantation than the ATG-F and this might have affected blood ATG concentration on day 0 and thereby affected the strength of *in vivo* T-cell depletion.

In conclusion, ATG-G seemed to have a stronger immunosuppressive activity than the ATG-F with a dose ratio of 1 : 2.5. The current approved dose of ATG-G in Japan (2.5 mg/kg/day for 4 days) may not be appropriate

and should be reduced at least in HLA-matched transplantation. Recent report from Korea suggested that low dose ATG-G at 1.25 mg/kg on days -3 and -2 was effective to prevent severe acute GVHD in HLA-mismatched unrelated peripheral blood stem cell transplantation[21]. Also, the timing of the ATG administration should be further studied, since the administration of ATG closer to the day of transplantation induces stronger *in vivo* depletion of donor T-cells.

Conflict-of-interest disclosure: All authors declare no competing financial interests.

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Table 1. Patient characteristics.

	Age/Sex	Grade	Prior IST	Regimen	Donor	Source	Recipient CMV	Donor CMV	acute GVHD	chronic GVHD
ATG-F-1	18/F	Severe	2	FLU-CY-ATG-TBI2	1 locus mismatched father	BM	Positive	Positive	None	None
ATG-F-2	35/M	Very severe	0	FLU-CY-ATG	matched sibling	PB	Positive	Positive	None	None
ATG-F-3	25/F	Very severe	0	FLU-CY-ATG-TBI2	1 locus mismatched sibling	PB	Positive	Positive	None	Limited
ATG-F-4	18/F	Severe	1	FLU-CY-ATG	matched sibling	BM	Positive	Positive	None	Extensive
ATG-G-1	46/M	Severe	1	FLU-CY-ATG-TBI2	1 locus mismatched sibling	BM	Positive	Positive	Grade I	Extensive
ATG-G-2	58/F	Severe	2	FLU-CY-ATG-TBI2	matched unrelated donor	BM	Positive	Negative	Grade I	Limited
ATG-G-3	48/F	Very severe	2	FLU-CY-ATG	matched sibling	PB	Positive	Negative	None	N.A.

IST: immunosuppressive therapy, FLU: fludarabine, CY: cyclophosphamide, ATG: antithymocyte globulin, TBI2: total body irradiation at 2 Gy
BM: bone marrow, PB: peripheral blood, GVHD: graft-versus-host disease

Figure legends.

Figure 1. Serial changes in the number of cytomegalovirus (CMV)-antigenemia cells per 2 slides in the ATG-F group (A) and ATG-G group (B). All three patients in the ATG-G group developed positive antigenemia, whereas 2 of the 4 patients in the ATG-F group were persistently negative for CMV antigenemia.

Figure 2. Immune recovery on day 60 after transplantation. Closed triangles and open circles indicate the number of cells (/μL) in the peripheral blood on day 60 after transplantation in the ATG-F and ATG-G groups, respectively.

Figure 1.

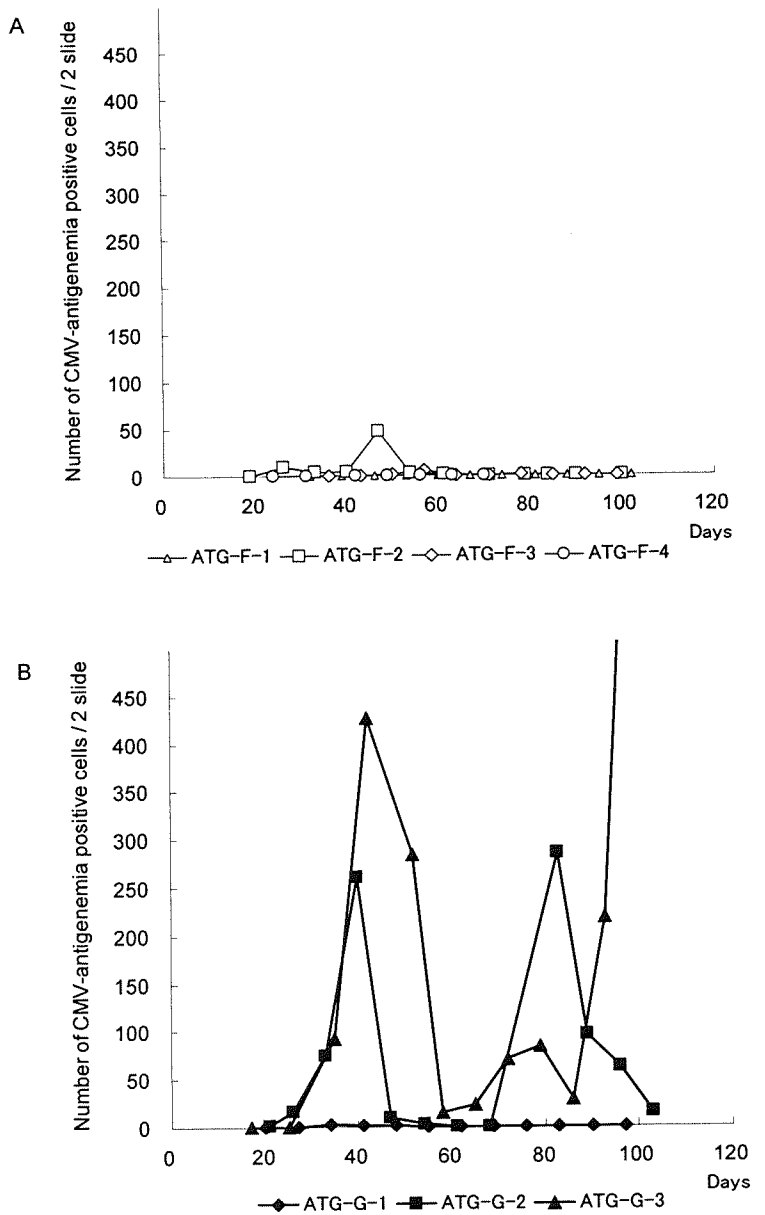
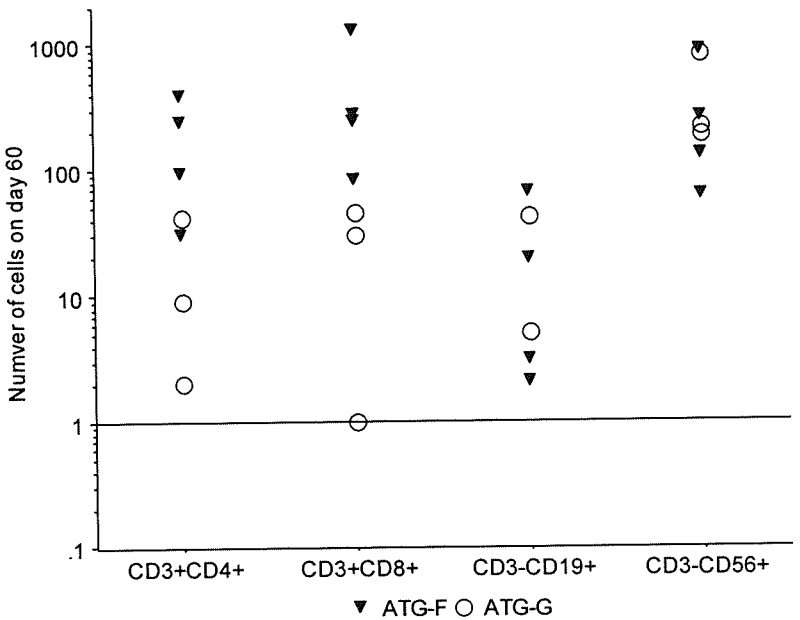


Figure 2



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CASE REPORT

Cord blood transplantation using minimum conditioning regimens for patients with hematologic malignancies complicated by severe infections

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Abstract Patients with severe infections are thought to be ineligible for cord blood stem cell transplantation (CBT) because the conventional 5–6 day-conditioning regimens potentially makes them susceptible to fatal infections by the time neutrophil engraftment occurs. Two patients were treated with minimum conditioning regimens consisting of 30 mg/m^2 fludarabin (Flu) and 2 g/m^2 cyclophosphamide (CY) on day-1 and total body irradiation (TBI) of 2 or 4 Gy on day −1 or 0 followed by single unit CBT. The reasons for adopting such weak regimen were febrile neutropenia due to the rejection of the first cord blood (CB) graft given to a patient with follicular lymphoma resistant to chemotherapy and pulmonary aspergillosis in another patient with AML who relapsed after CBT. The AML patient received 40 mg/m^2 of melphalan on day-2 to reduce the leukemia burden. Both patients achieved 100% donor chimerism by day 19 and day 20 after CBT without an apparent exacerbation of the infections and remained in remission at 23 and 18 months after the CBT. These findings suggest that the 1–2 day regimens excluding antihuman thymocyte globulin may be sufficiently potent to ensure engraftment of CB in immunocompromised patients and safely administered even when patients are complicated by active infections.

Keywords Cord blood transplantation · Active infection · Minimum intensity conditioning regimen

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1 Introduction

Cord blood (CB) is becoming a major source of allogeneic hematopoietic stem cell transplantation [1, 2]. The success of reduced intensity CB transplantation has accelerated the use of CB for treatment of aged patients with hematologic malignancies [3]. However, patients complicated by severe documented infections are still considered ineligible for cord blood transplantation (CBT) even if reduced intensity regimens are adopted because the preconditioning causes severe neutropenia which usually lasts until day 20 after transplantation [2, 3] and exacerbates infections leading to treatment related-death. As a result, some patients with hematologic malignancies who failed to achieve remission after chemotherapy or those who failed to engraft after allogeneic stem cell transplantation cannot benefit from CBT.

One possible measure to solve this problem is to shorten the time for preconditioning in addition to reducing the intensity. Since most conventional preconditioning regimens take more than 4 days, they need to be started at least 5 days prior to the day of transplantation [4]. Shortening the time for preconditioning to 1 or 2 days may help patients to survive a neutopenic period from the start of preconditioning to neutrophil engraftment. Goggins et al. used a 1-day conditioning regimen consisting of fludarabin (Flu), alemtuzumab and cyclophosphamide (CY) to treat five leukemia patients with allogeneic peripheral blood stem cell transplantation (PBSCT) and observed stable engraftment in three patients. A similar 1-day regimen consisting of Flu, CY and antihuman thymocyte globulin (ATG) was used to treat a myelodysplastic syndrome (MDS) patient with a second allogeneic PBSCT (K. Mochizuki et al., in preparation). The patient suffered from a high fever suggestive of bacteremia due to

persistent neutropenia following the rejection of the first PBSC graft. The second PBSC of another HLA-identical sibling from the original donor successfully engrafted and the patient has been in remission for more than 4 years. However, all of these cases used PBSC grafts containing a high number of hematopoietic stem cells as well as T cells which are thought to be helpful to accelerate the engraftment of donor stem cells and rapid neutrophil recovery. It is still unclear whether CB can engraft after such a very weak regimen and eventually rescue neutropenic patients complicated by severe infections.

This report describes two patients with a devastating condition who were successfully treated with a minimum intensity regimen of 1–2 days followed by single unit CBT.

2 Patients

2.1 Patient 1

In January 2005, a 56-year-old man was diagnosed to have a clinical stage IV follicular lymphoma. He achieved only PR after standard chemotherapy consisting of rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone and was refractory to other chemotherapy regimens for salvage. He underwent CBT following a reduced conditioning regimen consisting of cladribine, CY and 4 Gy of total body irradiation (TBI). His neutrophil count remained at 0 on day 21 after the CBT. A chimerism analysis of the bone marrow cells preformed on the same day revealed 100% cells to be recipient-type, thus indicating graft rejection. There was no sign of autologous hematologic recovery and a high fever persisted. There was no sign of an autologous hematologic recovery and a high fever persisted despite the administration of meropenem 1.0 g twice daily and micafungin 300 mg daily. The patient's CRP rose to 25.9 mg/dl on day 25. On day 27 after CBT, he received 30 mg/m^2 Flu and 2 g/m^2 CY followed by 2 Gy of TBI in the morning of the next day. HLA 2 locus-mismatched CB containing 2.6 $\times$ 10^7/kg cells and 9.8 $\times$ 10^6 CD34$^+$ cells/kg was infused 13 h after the completion of CY infusion. Clinical data including HLA alleles of the patient, the first CB donor, and the second CB donor are shown in Table 1. Tacrolimus was given from day-1 for prophylaxis of GVHD. The high fever started abating on day 16 after the second CBT and his neutrophil count surpassed 0.5 $\times$ 10^9/l on day 19. A chimerism analysis performed on day 26 revealed the 100% of the peripheral blood leukocytes were donor-type. Although grade I GVHD occurred, it resolved without treatment. CT scanning on day 33 after the second CBT showed a marked reduction of cervical lymph node swelling in comparison to that at 29 days before the first CBT (Fig. 1). He remains well in partial remission 30 months after the second CBT.

Table 1 Clinical data and HLA alleles of the patients and cord blood donors

	Sex	Blood type	HLA-A	HLA-B	HLA-DR
Patient 1	M	O+	0206/3303	3901/4403	1302/1501
First CB for patient 1	M	O+	0201/3303	3501/4403	1302/1501
Second CB for patient 1	M	A+	1101/3303	3901/4403	0803/1501
Patient 2	F	A+	2402/–	3501/4001	0901/1302
First CB for patient 2	M	AB+	0201/2402	3501/4006	0901/1302
Second CB for patient 2	M	B+	2402/–	4001/4006	0901/1501

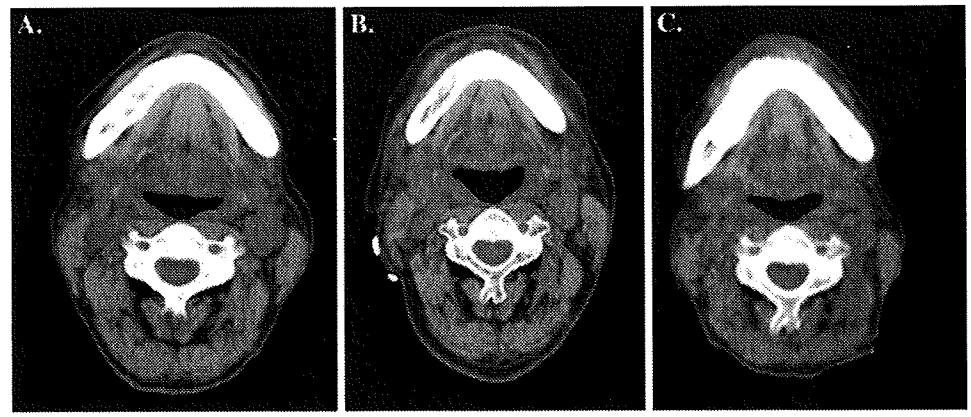

Fig. 1 Changes in the cervical lymphoma lesions after CBT in patient 1. CT scan on 23 months after the second CBT showed a marked reduction in size of the cervical lymph nodes in comparison to those before the first and the second CBT

A. 29 days before the first CBT B. 25 days after the first CBT C. 23 months after the second CBT

2.2 Patient 2

In April 2005, a 66-year-old female was diagnosed to have AML evolving from MDS. Chemotherapy consisting of idarubicin (IDA) and cytosine arabinoside (Ara-C) failed to induce remission and severe pancytopenia persisted. She underwent CBT following a conditioning regimen with fludarabine, melphalan, rabbit ATG and 4 Gy of TBI. The CB was 2-loci mismatched and contained 2.9×10^7/kg cells. Engraftment was confirmed on day 18 and she achieved complete remission. However, the AML relapsed in 18 months after the CBT. Remission induction with IDA and Ara-C only induced marrow hypoplasia with 33% residual leukemia cells. On day 18 of the chemotherapy, invasive aspergillosis developed in the left lung. Liposomal amphotericin B, 2.5 mg/kg daily, was administered from the same day without any appreciable effects. The neutrophil count remained at 0 on day 22 of the chemotherapy. She received melphalan 40 mg/m^2 to reduce leukemic cell burden, followed by 30 mg/m^2 Flu and 2 g/m^2 CY on the next day. In the morning of the following day, she received 4 Gy of TBI and underwent a second CBT 12 h after the completion of CY infusion. The CB was 2-loci mismatched, and contained 2.9×10^7/kg cells and 1.9×10^6 CD34$^+$ cells/kg. HLA alleles of the patient, the first CB donor, and the second CB donor are shown in Table 1. Tacrolimus was given from day-1 for prophylaxis of GVHD. Liposomal amphotericin B was switched to voliconazole, 4.0 mg/kg daily, on day 48 after the second CBT due to a rise in the creatinine level. Although her pulmonary aspergillosis was transiently exacerbated on day 6 after the second CBT, the high fever abated on day 17 and engraftment of donor cells was confirmed on the same day. The aspergillosis lesion was encapsulated with time after the second CBT (Fig. 2).

She underwent a left upper lobectomy on day 113 and presently remains in CR at 24 months after the second CBT.

3 Discussion

Treatment of the patients with hematologic malignancies complicated by severe neutropenic infections with no hope of prompt hematologic recovery is challenging. Although immunoablative conditioning followed by allogeneic stem cell transplantation is the only measure to rescue patients with such devastating conditions, this treatment may also tend to sometimes hasten the patients' death by aggravating the preexisting infections. Even if reduced intensity regimens are adopted, severe neutropenia which lasts from the day of preconditioning until 2–3 weeks after SCT greatly increases the risk of infectious death [3, 5, 6]. In order to solve this dilemma, Goggins et al. pioneered a very weak conditioning regimen, known as the 1-day regimen [7]. They treated five infirmed patients with 30 mg/m^2 Flu, 2 g/m^2 CY, 20 mg/kg alemtuzumab, TBI 2 Gy on day-1 and infused PBSC from family donors who were HLA 1–3 loci mismatched. Engraftment occurred in three patients, two of whom achieved long-term remission. According to their protocol, an MDS patient who suffered febrile neutropenia due to rejection of the first PBSCT was treated with Flu (30 mg/m^2), CY (2 g/m^2), horse ATG (15 mg/kg) and TBI (2 Gy) followed by PBSCT from a second HLA-identical sibling donor. The neutrophil count promptly recovered and the patient achieved complete donor chimerism. This experience indicated that the alemtuzumab in the 1 day regimen can be replaced with low dose ATG and that the minimum conditioning regimen coupled with PBSCT from a second donor can overcome the rejection after SCT.

Cord blood transplantation is associated with a higher incidence of engraftment failure [8–12] and a slower neutrophil recovery [2, 9, 13] than BMT or PBSCT due to the low number of hematopoietic stem cells and mature T cells in the CB graft. The disadvantages of CBT has precluded the use of CB for treatment of patients with very low intensity regimens for allogeneic stem cell transplantation such as 2 Gy TBI alone [14] or ATG + total lymphoid

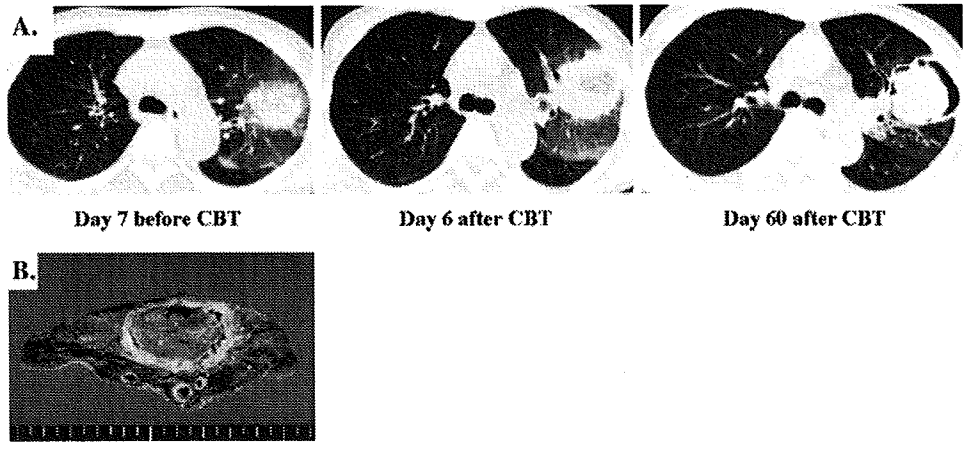

Fig. 2 Pulmonary aspergillosis lesion of patient 2. **a** Changes in the CT findings before and after CBT. **b** *Left upper* lung resected on day113 after CBT

irradiation regimens [15]. However, there were no options other than CBT for the two current patients because they did not have matched family donors and could not afford to wait until an HLA-matched unrelated donor was available. ATG was not included in the conditioning regimen for those patients because they could have succumbed to their infections which became exacerbated by the administration of ATG. Despite their devastating conditions and the elimination of ATG from the conditioning regimen, both patients achieved engraftment of CB without any apparent exacerbation of their infections or the development of severe GVHD. Therefore, in vivo purging of T cells using anti-T cell antibodies may not be a prerequisite for engraftment of CB after the 1–2-day regimen. However, it should be noted that both patients had been previously treated with conditioning regimens for allo-SCT. Prior conditioning regimens used for the first CBT may therefore be necessary for patients to take CB following such a minimum conditioning regimen. Other reduced-intensity regimens have been successfully used as preconditioning for a second transplantation using CB to treat graft rejection after allo-SCT [16–20]. However, all such regimens were administered for over 5 days and were not as weak as the regimens we used for the above described two patients.

Sustained engraftment of CB after the weak regimen in the current patients may therefore have important implications in the management of patients with hematologic malignancies refractory to chemotherapy. Patients who fail chemotherapy often suffer from severe infections due to persistent neutropenia and are therefore excluded as candidates for hematopoietic stem cell transplantation, particularly CBT, which is associated with delayed neutrophil recovery. Following very weak preconditioning, the patients not only circumvented life threatening infections but also achieved hematologic remission possibly with the help of the graft-versus-leukemia/lymphoma effects of CBT. CB can be utilized for patients with severe complications because of its easy accessibility and prompt availability [21]. Therefore, CBT following the minimum intensity conditioning may provide a chance to achieve complete chimerism in patients suffering from severe infections associated with profound neutropenia due to graft rejection or chemotherapy for leukemic relapse after the first allo-SCT.

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Anti-Moesin Antibodies in the Serum of Patients with Aplastic Anemia Stimulate Peripheral Blood Mononuclear Cells to Secrete TNF-α and IFN-γ[1]

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Moesin is an intracellular protein that links the cell membrane and cytoskeleton, while also mediating the formation of microtubules and cell adhesion sites as well as ruffling of the cell membrane. To determine the roles of anti-moesin Abs derived from the serum of patients with aplastic anemia (AA) in the pathophysiology of bone marrow failure, we studied the expression of moesin on various blood cells and the effects of anti-moesin Abs on the moesin-expressing cells. The proteins recognized by anti-moesin mAbs were detectable on the surface of T cells, NK cells, and monocytes from healthy individuals as well as on THP-1 cells. The peptide mass fingerprinting of the THP-1 cell surface protein and the knock-down experiments using short hairpin RNA proved that the protein is moesin itself. Both the anti-moesin mAbs and the anti-moesin polyclonal Abs purified from the AA patients' sera stimulated THP-1 cells and the PBMCs of healthy individuals and AA patients to secrete 60–80% as much TNF-α as did LPS 100 ng/ml. Although the polyclonal Abs induced IFN-γ secretion from the PBMCs of healthy individuals only when the PBMCs were prestimulated by anti-CD3 mAbs, the anti-moesin Abs were capable of inducing IFN-γ secretion from the PBMCs of AA patients by themselves. Anti-moesin Abs may therefore indirectly contribute to the suppression of hematopoiesis in AA patients by inducing myelosuppressive cytokines from immunocompetent cells. ***The Journal of Immunology,*** **2009, 182: 703–710.**

Acquired aplastic anemia (AA)[4] is a syndrome characterized by pancytopenia and bone marrow (BM) hypoplasia (1). The T cell-mediated suppression of hematopoiesis is considered to be the most important mechanism responsible for the development of this syndrome because approximately 70% of AA patients respond to immunosuppressive therapy, such as antithymocyte globulin and cyclosporine (2, 3). In addition to a large body of evidence for T cell involvement in the pathogenesis of AA (4–7), recent studies have revealed the presence of Abs specific to self-Ags in the serum of AA patients (8–11). Although some of these Abs are directed toward Ags that are abundant in hematopoietic cells (e.g., kinectin (Ref. 8) and DRS-1 (Ref. 9)), their roles in the pathophysiology of AA are unclear.

Of the various autoAbs detected in the autoimmune diseases, some are known to exhibit stimulatory effects on the target cells rather than inhibitory effects, such as anti-thyroglobulin Abs in Basedow's disease (12) and anti-desmoglein Abs in pemphigus vulgaris (12, 13). The autoAbs specific to platelet-derived growth factor receptors in patients with scleroderma and those with extensive chronic graft-vs-host diseases trigger an intracellular loop, involving Ha-Ras-ERK 1 and 2 (ERK 1/2)-reactive oxygen species (Ha-Ras-ERK 1/2-ROS), and augment collagen gene expression as well as myofibroblast phenotype conversion of normal human primary fibroblasts (14, 15). The anti-proteinase 3 Abs detected in Wegener's granuloma stimulate monocytes through the binding of cell surface proteinase 3 to secrete IL-8 (16). The autoAbs detected in AA patients may also be involved in the pathophysiology of BM failure by way of other mechanisms than the direct toxicity against the hematopoietic cells, though there has been no evidence for such functional autoAbs in AA patients.

We previously demonstrated that Abs specific to moesin, a membrane-cytoskeleton linker protein in the cytoplasm, were detectable in approximately 40% of AA patients (11). Moesin is an intracellular protein that links the cell membrane and cytoskeleton, and mediates the formation of microtubules and cell adhesion sites as well as ruffling of the cell membrane (17). On the other hand, some reports have identified molecules that were recognized by anti-moesin mAbs on the surface of blood cells such as T cells and macrophages (18, 19). Because these immune cells are an important source of myelosuppressive cytokines such as TNF-α and IFN-γ, it is conceivable that anti-moesin Abs in AA patients may bind such moesin-like molecules on these immune cells and affect the cytokine secretion from these cells.

To test these hypotheses, we studied the expression of moesin on blood cells and the effects of anti-moesin Abs on the moesin-expressing

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[4] Abbreviations used in this paper: AA, aplastic anemia; BM, bone marrow; pAb, polyclonal Ab; PB, peripheral blood.

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