Risk factor analysis of vasovagal reaction from blood donation

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ABSTRACT

Background: Vasovagal reaction (VVR) is the most frequent side effect at blood collection sites.

Aims: To protect donors, factors contributing to VVR were analysed.

Materials and methods: Complications following whole blood and apheresis donations have been recorded and accumulated by the Japanese Red Cross Tokyo Blood Centre. A dataset of 43,948 donors who had no complications was prepared as a control by randomly selecting days in each season in the 2006 and 2007 fiscal years. Factors contributing to 4924 VVR incidents in the 2006 and 2007 fiscal years were analysed by univariate and multivariate logistic regression.

Results: The age, weight, body mass index (BMI), predonation systolic and diastolic pressure, and circulating blood volume were lower, and the pulse was higher, for the VVR group compared to the control group ($p < 0.0001$). The VVR group had more female donors, less sleep, and more time since a meal than the control. In multivariate analysis, significant risk factors for 400 ml whole blood donors, which are the majority of donors, were an age <50 years, being female, a BMI <25, pulse ⩾90/min, sleep duration <8 h, the time after eating ⩾4 h, a first time donation and circulating blood volume of <4.3 l. Sleep duration of <6 h was shown to be a VVR risk as much as a first time donation.

Conclusion: From our analysis, the amount of sleep obtained the previous night should be considered at the reception of donors.

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1. Introduction

The blood supply depends entirely on volunteers. Blood collection, though, sometimes causes adverse reactions. The most frequent adverse reaction with blood collection is a vasovagal reaction (VVR), a systemic prefaint reaction, and it is usually mild and transient. A severe VVR including loss of consciousness or syncope can cause injury, from bruising to bone fractures, losing teeth or a brain injury. The incidence of VVR from blood collection in a Japanese Red Cross (JRC) blood centre had been reported to be between 0.83% and 4.17% depending on the sex and donation type [1]. The high risk groups for VVR are generally reported to be young, female and first-time donors [2–6].

The JRC blood centres receive around 5 million donations of whole blood (WB) or apheresis components each year, providing 100% of the Japanese blood supply. The JRC Tokyo Blood Centre receives around 11% of all the donations in Japan. Since we experienced a fatal injury associated with syncope after a donation in 2005, we began multiple analyses that included setting up a database for

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donors who did not experience adverse reactions. This report describes the risks of VVR, using the donor complication data gathered for two fiscal years, compared with information on donors without adverse reactions randomly collected during the same period as a control.

2. Materials and methods

2.1. Collection site procedures

In Japan we have 200 ml WB (WB200), 400 ml WB (WB400), apheresis platelet and plasma collection. Donors who are 16 or 17 years old had been able to make only WB200 donations until April 2011, at which time we began to accept 17 year old male donors for WB400. The WB200 group has predominantly consisted of young, first-time donors or of low weight donors. All donations are allogeneic, from healthy individuals.

Platelets and plasma are collected with one of three apheresis systems, the Trima (Gambro BCT, Lakewood, CO), TerusysS (Terumo, Tokyo, Japan) or Haemonetics CCS (Haemonetics, Braintree, MA). The circulating blood volume (BV, l) is calculated according to Ogawa et al. [7]:

$$BV = 0.168H^3 + 0.050W + 0.444 \text{ for an adult male, and}$$

$$BV = 0.250H^3 + 0.0625W - 0.662 \text{ for an adult female,}$$

where H is the height (m) and W is the weight (kg). The collecting volume of plasmapheresis is between 300 and 600 ml, depending on the body weight, and the volume for platelet collection is 400 ml or less.

Adverse reactions which occur at the collection sites are managed by the collection staff, including physicians. Severe cases are sent to hospital for consultation and treatment. All donors are instructed to contact the blood centre if they experience problems or have concerns about their health after making the donation. We keep a systematic record of events that occur at the time of the donation, or that are reported later, including reports when donors receive outside medical care.

2.2. VVR group

Donor adverse reactions were recorded in our central data base system. When a donor presented symptoms including weakness, pallor, yawning, cold sweat, nausea, vomiting, fainting, convulsion and incontinence, with or without lower systolic blood pressure (BP) or lower pulse rate compared to those recorded at reception, the nurse recorded the complication as VVR, and more information about the timing, place and accompanying fainting was requested to add into the records.

From April 1, 2006 to March 31, 2008, during which time we accepted 1,119,716 donations, we recorded 13,320 donor adverse reactions (1.09%). Selecting records with the primary or secondary complication being VVR, and with sufficient information, we compiled a VVR group of 4924.

2.3. Control group

Days were selected randomly for each season over two years, and for donors who had been accepted on those days, BP, pulse rate before and after the donation, sleep duration, and time since eating were entered into a data base from the paper form which had been recorded at reception. The data was later combined with other information from the central donor data system, such as age, sex, number of donations, donation volume, donation site and time. By eliminating the donations with adverse reactions from the data, we prepared a control dataset of 43,948 donations.

2.4. Statistical analysis

Descriptive statistical analysis was performed to assess donor baseline characteristics, BMI, predonation pulse, predonation systolic and diastolic BP, sleep duration, time since eating, and type of donation. The group was dichotomised for BMI, pulse, BP, hours since a meal and sleep duration at reported or clinically relevant figures. The 2-sided chi-square test was used for categorical variables, and the 2-sided Wilcoxon rank sum test was used for continuous variables.

Furthermore, VVR rates for different subgroups were compared by calculating odds ratios (ORs) and 95% confidence intervals (CIs). A multivariate logistic regression analysis was performed to identify demographic variables that were independently associated with VVR after donations using SAS software (Version 9.1.3, SAS Institute, Inc., Cary, NC). In the multivariate logistic analyses, ORs were adjusted for age group, BMI group, pulse group and systolic BP group.

3. Results

3.1. VVR incidents

The location data was obtained for 4908 of the 4924 VVR incidents studied: 87.1% of the VVR occurred in the collection room, followed by 10.6% in the refreshment area, 1.2% outside of the collection sites, 0.8% on the way from the collection room to the refreshment area, and 0.4% in the restroom.

With the timing data obtained for 4818 incidents, 2621 (54.4%) of the VVR occurred during the collection, followed by 28.6% after the collection and 9.8% while in the refreshment area. There were 67 incidents of VVR which occurred after blood was collected and the donor left the site. Other VVR incidents associated with our procedure for a haemoglobin test, requiring venipuncture, included 4.0% after the haemoglobin test and before the blood collection, 1.6% during the haemoglobin test and 0.3% before the test.

The percentages of the VVR group whose donation site was a mobile bus, temporary donation site (mobile setup) or donation room were 34.1%, 5.0% and 60.9%, respectively, while the donation site ratio of the control group was 13.7%, 9.3% and 77.0%, respectively ($p < 0.0001$).

3.2. Characteristics of donors

The characteristics of the VVR and the control groups were significantly different ($p < 0.0001$) in: age (mean 28.3 vs. 34.9 years), height (163.9 vs. 166.4 cm), weight (58.2 vs. 62.8 kg), BMI (21.6 vs. 22.6), predonation pulse (77.7 vs. 76.1 beats/min), predonation systolic BP (116.6 vs. 119.5 mmHg), predonation diastolic BP (69.2 vs. 71.7 mm Hg) and circulating BV (4.04 vs. 4.33 l). The mean of the circulating BV was 4.30 l. The VVR group consisted of significantly ($p < 0.0001$) more female donors (50.5% vs. 36.9%), first time donors (38.2 vs. 11.2%), and donors who had more than 4 h since their last meal (43.1% vs. 36.0%). The VVR group also had more donors who had less sleep the night before ($p < 0.0001$), as shown in Fig. 1.

When the VVR group was divided according to the timing data, into 'VVR before or during collection' and 'VVR after donation' subgroups, the first subgroup was significantly younger (age 27.7 vs. 29.2 years old), shorter (163.4 cm vs. 164.6 cm), lighter (57.3 kg vs. 59.6 kg) and had a higher ratio of female donors compared to the 'VVR after donation' subgroup. The 'VVR after donation' group was also significantly younger, shorter, lighter, and had a higher ratio of female donors compared to the control group. The donor characteristics of the timing subgroups were influenced by the ratio of female donors with VVR from apheresis. With apheresis donation, 82% of the VVR occurred before or during the collection, and 76% of the VVR from apheresis were female donors. WB400 donors were the majority (76%) of the 'VVR after donation' subgroup, while they were 44% of the 'VVR before and during collection' subgroup, less than the apheresis donors (49%). The sleep duration, time from eating and first time donor ratio were not significantly different between the two subgroups.

3.3. Subgroup analysis by sex

For male donors, the VVR group was significantly younger (mean 28.2 years old vs. 36.6 years old), shorter (170.4 cm vs. 171.0 cm), lighter (63.8 kg vs. 68.3 kg), had a lower BMI (22.0 vs. 23.3), and a lower circulating BV (4.5 l vs. 4.7 l) ($p < 0.0001$). The VVR group also had significantly lower predonation systolic BP (121.9 mmHg vs. 124.2 mmHg) and diastolic BP (71.5 mmHg vs. 74.5 mmHg) ($p < 0.0001$).

For female donors, the VVR group was significantly younger (mean 28.3 years old vs. 31.9 years old), shorter (157.5 cm vs. 158.6 cm), lighter (52.7 kg vs. 53.5 kg), and had a lower circulating BV (3.6 l vs. 3.7 l) ($p < 0.0001$). The VVR group also had a significantly higher pulse (79.2 vs. 76.3 beats/min) ($p < 0.0001$).

In the multivariate analysis, the female subgroup showed a higher risk for ages 18 and 19 (OR 3.1, 95%CI 2.5–3.8) and for those aged in their twenties (OR 1.7, 95%CI 1.4–2.0), compared to those aged ⩾50. The male subgroup had a high OR in every age group <50 years, especially for ages 18 and 19 (OR 19.6, 95%CI 14.9–25.8). The male subgroup also showed an association with BMI with a higher OR for a BMI <25 compared to those of ⩾25. In both subgroups a predonation pulse ⩾90/min showed a high OR, at 1.6 (95%CI 1.4–1.8) for females and

1.2 (95%CI 1.1–1.4) for males, compared to those with <90/min. First time donors showed a high risk, with an OR of 2.3 (95%CI 2.0–2.5) for females and an OR of 5.9 (95%CI 5.3–6.4) for males, compared to other donors. Sleep of less than 6 h was associated with a high OR in both subgroups compared to those with ⩾8 h, at 3.6 (95%CI 3.2–4.1) for females and 5.1 (95%CI 4.5–5.9) for males. A time from the last meal of ⩾4 h was a risk for VVR in both subgroups, with an OR of 1.24 (95%CI 1.13–1.35) for females and 1.64 (95%CI 1.48–1.82) for males. The risk was significantly lower with WB200 than WB400 donations in both subgroups, while apheresis had shown a higher risk for females with an OR of 4.42 (95%CI 3.9–5.1), but lower for males with an OR of 0.7 (95%CI 0.5–1.0). A diastolic BP <70 mmHg was shown as a low risk factor in both subgroups.

3.4. Subgroup analysis by donation types

The characteristics of donation type subgroups are shown in Table 1. In every donation type, the VVR group was younger, lighter, had a smaller BMI, smaller circulating BV, and had more donors with less than 6 h sleep. Only for the 200WB donations were there more male donors in the VVR group.

The multivariate analyses for each subgroup, adjusted for age, sex, BMI, pulse and systolic pressure, are shown in Table 2. A younger age, shorter sleep duration, first time donation and circulating BV <4.30 l were the common risk factors for all three subgroups. Also, a predonation diastolic BP lower than 70 mmHg was a significant low risk factor for VVR for every subgroup.

For the WB200 donations, the age groups of 16 and 17 year olds, 18 and 19 year olds and of those in their twenties and thirties showed a high adjusted OR compared to those aged ⩾50 years. A sleep duration <8 h was shown to be a risk factor, especially when it was <6 h.

For WB400 donations, which make up the majority of our collections, a younger age compared to the age group of ⩾50 years showed significantly higher risk. Other significant high risk factors were being female, a BMI <25, a predonation pulse ⩾90 beats/min, sleep duration <8 h, especially when it is <6 h, time since eating ⩾4 h, a first-time donation and a circulating BV <4.30 l. A predonation systolic BP lower than 100 mmHg and a diastolic BP lower than 70 mmHg were significant low risk factors for VVR.

For apheresis donations, the significant factors for high risk of VVR were ages 18 and 19 years, being female, a BMI <25, a predonation pulse ⩾90 beats/min, sleep duration <8 h, especially when it is <6 h, time after eating ⩾4 h, a first-time donation and a circulating BV <4.30 l.

3.5. Risk analysis of syncope/fall

There were 75 syncope/fall incidents in the dataset. The majority, 74 incidents, were a consequence of VVR and the other case had a syncope following the side effect of haematoma. When they were divided by the timing data, 21 cases occurred before and during collection, and 53 cases had the syncope/fall incidents after the donation. The incident rate as a proportion of VVR was shown to
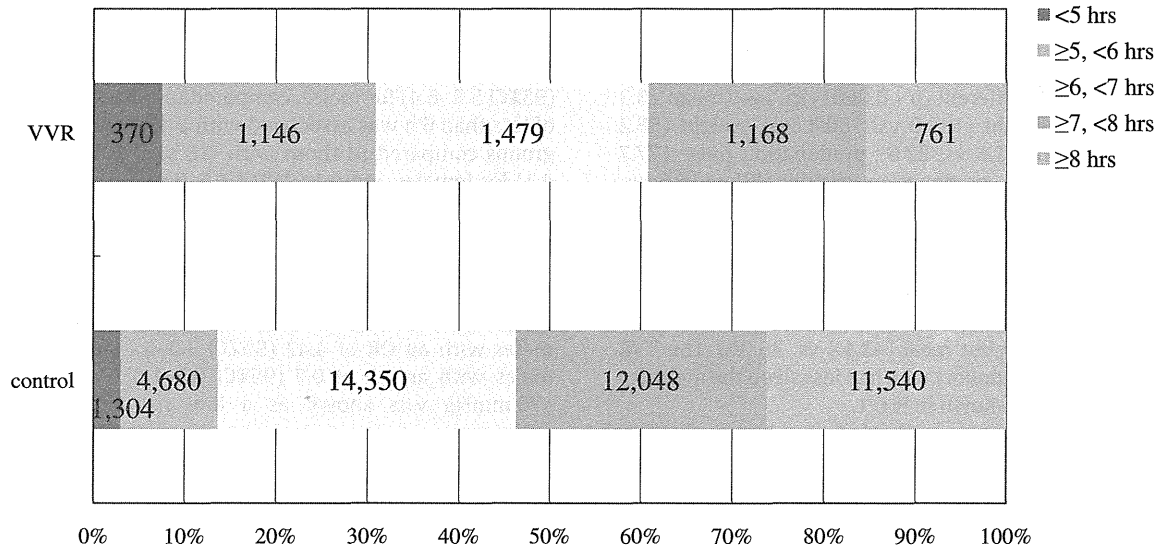
Fig. 1. Sleep duration for the VVR and control groups. The VVR group had significantly less sleep compared to the control group ($p < 0.0001$).

Table 1
Characteristics of the VVR and control group for donation type subgroups.

Variables	200 mL Whole blood		400 mL Whole blood		Apheresis	
	VVR (mean ± SD)	Control	VVR (mean ± SD)	Control	VVR (mean ± SD)	Control
	$n = 344$	$n = 5789$	$n = 2668$	$n = 24{,}602$	$n = 1637$	$n = 13{,}557$
Age (years old)	23.8 ± 7.5***	31.1 ± 12.5	28.0 ± 9.5***	36.1 ± 12.0	30.6 ± 11.0***	34.2 ± 11.2
Height (cm)	158.5 ± 6.9	158.4 ± 6.6	166.9 ± 7.7***	168.6 ± 7.5	159.7 ± 7.7***	166.0 ± 8.3
Weight (kg)	49.4 ± 6.7***	50.8 ± 7.6	61.6 ± 8.5***	66.2 ± 10.4	54.6 ± 8.9***	61.8 ± 11.2
BMI	19.6 ± 2.0***	20.2 ± 2.3	22.1 ± 2.6***	23.2 ± 3.0	21.4 ± 2.6***	22.3 ± 3.0
Predonation pulse (/min)	76.9 ± 11.4	76.3 ± 11.2	76.6 ± 12.3**	75.8 ± 11.6	80.0 ± 11.4***	76.5 ± 11.6
Predonation systolic pressure (mmHg)	110.1 ± 12.8	111.3 ± 14.2	120.0 ± 13.6***	123.3 ± 15.8	112.6 ± 14.0***	116.0 ± 14.7
Predonation diastolic pressure (mmHg)	67.2 ± 10.2	66.5 ± 10.5	71.0 ± 10.4***	74.0 ± 12.1	67.2 ± 10.3***	69.6 ± 11.5
Circulating blood volume (l)	3.34 ± 0.49**	3.51 ± 0.53	4.28 ± 0.53***	4.55 ± 0.61	3.76 ± 0.61***	4.27 ± 0.71
	(n)		(n)		(n)	
Sex Female/Male	280/64***	5115/674	847/1821***	5657/18,945	1249/388***	5445/8112
Sleep dilation (hours)						
< 6	127***	801	942***	3882	375***	1301
⩾ 6, < 7	84	1891	831	8206	483	4253
⩾ 7, < 8	83	1490	536	6510	471	4048
⩾ 8	50	1600	359	5990	308	3950
Time after eating (hours)						
<4/⩾4	148/196	2341/3441	1106/1562***	9377/15,192	753/884***	4058/9473
Donation status						
First-time/other	207/137***	1354/4435	1295/1373***	3353/21,249	178/1459***	233/13,324

** $p < 0.01$.
*** $p < 0.001$.

be higher after the donation (2.8% vs. 0.7%), suggesting the donors received closer care when the VVR symptom was noticed earlier. The multivariate analysis with the control group showed a trend similar to the VVR risk analysis, as the majority of syncope cases followed VVR. The risk of syncope was higher for the age group of 20–29 years with an OR of 3.7 (95%CI 1.1–12.3), being female with an OR of 1.9 (95%CI 1.2–3.1), sleep duration <6 h with an OR of 4.2 (95%CI 2.1–8.2), first time donors with an OR of 2.8

(95%CI 1.7–4.7), and circulating BV <4.3 l with an OR of 3.4 (95%CI 1.6–7.0). Donations other than WB200 also had a higher risk compared to WB200.

4. Discussion

As the only organization to collect blood for transfusion in Japan, the Japanese Red Cross has been trying to prevent adverse reactions from blood donation, as well as the side

Table 2
Donation subgroup analyses of VVR.

	Adjusted OR[a]	(95% CI)
200 ml Whole blood donation		
Age[b]		
16–17 year	11.86	(4.62–30.45)***
18–19 year	16.33	(6.40–41.69)***
20–29 year	11.16	(4.52–27.58)***
30–39 year	4.97	(1.95–12.68)***
Predonation diastolic pressure <70 mmHg	0.46	(0.36–0.59)***
Sleep duration[c]		
<6 h	5.84	(4.14–8.25)***
6–6.9 h	1.66	(1.15–2.38)***
7–7.9 h	2.00	(1.39–2.87)***
First-time donors	3.86	(3.03–4.90)***
Circulating blood volume <4.30 l	2.47	(1.45–4.20)***
400 ml Whole blood donation		
Age[b]		
18–19 year	12.13	(9.64–15.26)***
20–29 year	6.31	(5.13–7.76)***
30–39 year	3.23	(2.60–4.00)***
40–49 year	1.84	(1.45–2.34)***
Female	1.28	(1.17–1.40)***
BMI <25	1.67	(1.48–1.89)***
Predonation pulse ⩾90 beats/min	1.25	(1.11–1.40)***
Predonation systolic pressure <100 mmHg	0.71	(0.59–0.85)***
Predonation diastolic pressure <70 mmHg	0.77	(0.71–0.85)***
Sleep duration[b]		
<6 h	4.05	(3.56–4.60)***
6–6.9 h	1.92	(1.68–2.19)***
7–7.9 h	1.58	(1.37–1.82)***
Time after eating ⩾4 h	1.10	(1.01–1.19)*
First-time donors	4.21	(3.85–4.60)***
Circulating blood volume <4.30 l	1.69	(1.53–1.88)***
Apheresis donation		
Age[b]		
18–19 y	1.58	(1.18–2.11)**
Female	4.48	(3.95–5.07)***
BMI < 25	1.30	(1.08–1.57)**
Predonation pulse ⩾90 beats/min	1.90	(1.66–2.18)***
Predonation diastolic pressure <70 mmHg	0.86	(0.76–0.98)*
Sleep duration[c]		
<6 h	3.96	(3.33–4.69)***
6–6.9 h	1.54	(1.32–1.80)***
7–7.9 h	1.60	(1.37–1.87)***
Time after eating ⩾4 h	1.92	(1.73–2.14)***
First-time donors	5.10	(4.11–6.34)***
Circulating blood volume <4.30 l	2.06	(1.71–2.47)***

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.
[a] Odds ratio (OR) adjusted for age group, BMI group, pulse group and systolic blood pressure group.
[b] The risk was compared to age group of 50 years and older.
[c] The risk was compared to sleep duration of 8 h and more.

effects in recipients of transfusions. According to the nationwide donor records, adverse reactions have been reported for the fiscal years of 2007, 2008 and 2009 at the rate of 1.11%, 1.16% and 1.15%, respectively (JRC internal report). For the same years, in Tokyo the rates were 1.15%, 1.25% and 1.28%, respectively, with the majority of the incidences, 67.4%, 65.6% and 65.0%, respectively, consisting of VVR. This was in spite of the introduction of predonation hydration, following reports on decreasing such incidences by drinking water [8,9]. The analyses on risk factors of blood donation show that even a notably low risk population, males of higher weight, is not free from VVR.

Eder et al. [5] reported that regional practices could cause differences in incident rates. We also recognized an effect from the environment, as the VVR group more often consisted of donors at the mobile bus sites, which have a less spacious and less relaxed environment. The smaller ratio of the mobile setup locations for the VVR group (5.0%) compared to the control group (9.3%) may also reflect that the donors at mobile setup locations are often acquainted with each other, as these sites are usually at companies and community centres. As well, there was a tendency for a concentration of VVR cases in the peak afternoon time that often makes for a crowded and busy

environment. With age, it was reported that the reaction rates increase with decreasing age for teenaged donors [5,10]. We also observed an increase in risk with younger age in 400WB subgroup analysis, but it was not clear for the 200WB subgroup, presumably because the number is relatively small.

The weight of donors has been reported as a factor for VVR [2,3,8], but for this analysis we used BMI and circulating BV, which is calculated from the height, weight and sex of the donor. In our multivariate analyses we chose a BMI dichotomized at 25, for BMI $\geqslant 25$ is regarded as overweight, and we had a low OR for this group in the preliminary analysis. According to the national health and nutrition survey of Japan [11], 19% of women and 30% of men between 20 and 69 years old had a BMI $\geqslant 25$, so the ratio of overweight individuals was lower in our donor group, at 18%. The effect of BMI on the VVR risk was not significant for female donors, while it was significant for male donors, in sex subgroup analysis. BMI was also not significant for WB200 donors in donation subgroup analysis, probably because of the small size of the group with BMI $\geqslant 25$ in this subgroup, due to our practice of encouraging donors to switch from WB200 to WB400 when the donor's weight is more than 50 kg.

The circulating BV was dichotomized at the median, 4.30 l, and was a significant factor in every analysis. This is in agreement with Wiltbank et al. [6], whose report showed that a smaller BV is associated with a higher OR, especially for those with a BV <3.5 l. It was reported that greater blood loss was associated with presyncopal reactions [12]. We did not include volume loss as one of our factors, because the volume loss for the VVR group is rather small compared with donors without adverse reaction, as we stop the collection in cases when the donor has a symptom of VVR during the collection, which is when the majority of VVR occurs.

A predonation pulse $\geqslant 90$/min was a significant risk in analyses of the WB400, apheresis and sex subgroups. It is possible that stress or anxiety mounts before the donation, as a minority of donors had shown VVR even before the haemoglobin test. Our result is in accordance with the report by Wiltbank et al. [6], which had shown that compared to the group with a pulse of 65–90/min, >90 is a significantly high risk and <65 is a significantly low risk. We dichotomized the group at a pulse of 90/min, as the OR for a pulse of 65–90 and <65 were similar in our preliminary analysis.

The predonation systolic BP of <100 mmHg was shown to be a significant low risk factor in analyses of the WB400 subgroup. We dichotomized the group at 100 mmHg as the preliminary analysis had shown a similar OR for the groups with a BP of 100–140 and of >140 mmHg. The adjustment for the final analyses included age, pulse and systolic BP, as the younger the age, the lower the BP. There are reports that a higher systolic BP had shown a lower risk compared to normal BP, and lower BP was not significantly different [2,6]. It is possible that the demographics of the subjects caused the different results, as more than 9% of our analysed population had a BP <100 mmHg, while the report by Wiltbank et al. [6] showed it was 4%. A predonation

diastolic BP <70 mmHg compared with $\geqslant 70$ was a low risk factor in analyses for the sex subgroups, and every subgroup of donation type. There is a report showing a lower diastolic BP associated with a higher VVR rate, explaining that younger donors have lower BP [13]. This discrepancy with our results could be explained by our multivariate analyses being adjusted for factors including age. Also, there is a possibility that the donors who have a relatively low BP are used to having it, or that the BP change in VVR is relatively small.

In our practice, donors who had not eaten for a while, skipped their last meal or felt hungry were advised to come back after they had something to eat, or were sent for a snack and drink first. So the donors who stated at reception that they had gone $\geqslant 4$ h since their last meal had eaten at the time of venipuncture. There is a possibility that the donors who did not eat for $\geqslant 4$ h had been relatively busy and were under stress when they dropped into the donation site.

A short sleep duration was shown to be a significant risk factor for VVR, especially when it was less than 6 h. Tomasulo et al. [14] had shown that it was not necessary to consider the sleep history, since the sleep duration was not significantly different between the control donors and those with a reaction. In their study, the mean of the sleep duration for first time female donors was 7.8 h for the control and 7.7 h for those with a reaction, while for first time male donors the mean was 7.4 h for both. As our data for sleep hours is categorical and not numerical data, we cannot compare the sleep hours between their study and ours. Still, in our study the difference between the VVR group and the control group was significant. We have regular donors who work the night shift, so the sleep duration is not to be applied generally, but sufficient sleep is an important health factor, as Breslow and Enstrom [15] reported. The relatively high OR for groups with <6 h sleep should be considered when the donor belongs to a subgroup that is at higher risk, such as young, first-time donors, females or having a circulating BV <4.3 l.

The risk analyses of syncope reflected that for VVR, as the majority had been VVR cases. Young, female and first-time donors had been reported to be more likely in the syncopal group than general donors [16]. We have also shown that donors with a small BV and a short sleep duration should be added to the high risk groups.

Though our analyses of VVR was prompted by a serious accident related to syncope, it is difficult to apply all of the results directly to actual practice: It is essential to recruit young and first-time donors for the future of the blood supply, and also female donors, who made up 32.5% of the donors in Japan in the fiscal year of 2009. Well informed consent for blood donation is important [17]. To prevent serious consequences, the donors need to be informed about the posture to take when symptoms occur. Also, we should try to recruit donors from low risk groups.

The study showed the VVR risk factors and particularly the importance of the duration of sleep. From our analysis, the amount of sleep obtained the night prior to a blood donation should be considered at the reception of donors.

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Impact of graft-versus-host disease on outcomes after allogeneic hematopoietic cell transplantation for adult T-cell leukemia: a retrospective cohort study

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Impact of graft-versus-host disease on outcomes after allogeneic hematopoietic cell transplantation for adult T-cell leukemia: a retrospective cohort study

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Allogeneic hematopoietic cell transplantation (HCT) is an effective treatment for adult T-cell leukemia (ATL), raising the question about the role of graft-versus-leukemia effect against ATL. In this study, we retrospectively analyzed the effects of acute and chronic graft-versus-host disease (GVHD) on overall survival, disease-associated mortality, and treatment-related mortality among 294 ATL patients who received allogeneic HCT and survived at least 30 days posttransplant with sustained engraftment. Multivariate analyses treating the occurrence of GVHD as a time-varying covariate demonstrated that the development of grade 1-2 acute GVHD was significantly associated with higher overall survival (hazard ratio [HR] for death, 0.65; P = .018) compared with the absence of acute GVHD. Occurrence of either grade 1-2 or grade 3-4 acute GVHD was associated with lower disease-associated mortality compared with the absence of acute GVHD, whereas grade 3-4 acute GVHD was associated with a higher risk for treatment-related mortality (HR, 3.50; P < .001). The development of extensive chronic GVHD was associated with higher treatment-related mortality (HR, 2.75; P = .006) compared with the absence of chronic GVHD. Collectively, these results indicate that the development of mild-to-moderate acute GVHD confers a lower risk of disease progression and a beneficial influence on survival of allografted patients with ATL. (*Blood*. 2012;119(9):2141-2148)

Introduction

Adult T-cell leukemia (ATL) is a mature T-cell neoplasm that is causally associated with a retrovirus designated human T-cell leukemia virus type I (HTLV-I).[1-4] HTLV-I is endemic in southwestern Japan, sub-Saharan Africa, the Caribbean Basin, and South America.[3,4] In Japan, more than 1 million people were estimated to be infected with HTLV-I. Although the majority of HTLV-I–infected individuals remain asymptomatic throughout their lives, ~ 5% develop ATL at a median age of 40 to 60 years.[4,5]

ATL is categorized into 4 clinical variants according to its clinical features: smoldering, chronic, acute, and lymphoma types.[6] The acute and lymphoma variants of ATL have an extremely poor prognosis, mainly because of resistance to a variety of cytotoxic agents and susceptibility to opportunistic infections; the median

survival time is ~ 13 months with conventional chemotherapy,[7,8] although encouraging results have been recently reported with the use of novel agents such as mogamulizumab.[9-11]

Over the past decade, allogeneic hematopoietic cell transplantation (HCT) has been increasingly performed with the aim of improving dismal prognosis of patients who developed ATL.[12-18] Notably, some patients with ATL who relapsed after allogeneic HCT were shown to achieve remission only with the cessation of immunosuppressive agents, raising the question of whether the graft-versus-leukemia effect against ATL can be induced as part of graft-versus-host reaction.[19,20] In 1 study, among 10 patients who experienced relapse of ATL after transplantation and were withdrawn from immunosuppressive therapy, 8 developed graft-versus-host disease (GVHD), and 6 of them subsequently achieved

complete remission of ATL.[19] Similar observations have been rarely reported in other aggressive mature lymphoid neoplasms,[21] suggesting the unique susceptibility of ATL to graft-versus-host reactions. Recently, a combined analysis of 2 prospective studies including 29 ATL patients in total undergoing allogeneic HCT suggested that development of mild acute GVHD favorably affected overall survival and progression-free survival.[22] However, the impact of GVHD on the outcome of allogeneic HCT in ATL needs to be verified in a much larger cohort. We previously conducted a nationwide retrospective study to evaluate the current results of allogeneic HCT for ATL, and we confirmed that a substantial proportion of patients with ATL can enjoy long-term, disease-free survival after transplantation: the overall survival rate at 3 years among patients who received transplants in complete remission and not in complete remission was 51% and 26%, respectively.[23] Using the same cohort, we further evaluated the effects of acute and chronic GVHD on long-term outcomes of allografted patients with ATL.

Methods

Collection of data

Data on 417 patients with acute or lymphoma type ATL who had undergone allogeneic bone marrow, peripheral blood, or cord blood transplantation between January 1, 1996, and December 31, 2005, were collected through the Japan Society for Hematopoietic Cell Transplantation (JSHCT), the Japan Marrow Donor Program (JMDP), and the Japan Cord Blood Bank Network (JCBBN), the 3 largest HCT registries in our country; their roles were detailed previously.[23] The patients were included from 102 transplant centers; the data were updated as of December 2008. The study was approved by the data management committees of JSHCT, JMDP, and JCBBN, as well as by the institutional review boards of Kyoto University Graduate School of Medicine, where this study was organized.

Inclusion and exclusion criteria

Patients were included in the analysis if the following data were available: age at transplantation, sex of the recipient, donor type, stem cell source, agents used in the conditioning regimen and GVHD prophylaxis, the maximum grade and day of occurrence of acute GVHD, and the day of neutrophil recovery. Acute GVHD was reported according to the traditional criteria,[24] except that 1 patient was considered to have late-onset acute GVHD at day 133; neutrophil recovery was considered to have occurred when an absolute neutrophil count exceeded 0.5×10^9/L for 3 consecutive days after transplantation. Patients who missed any of these data (n = 37), who had a history of prior autologous or allogeneic HCT (n = 8), who had received an ex vivo T cell–depleted graft (n = 1), who experienced primary or secondary graft failure (n = 24) were excluded from the analysis. Because the association between the occurrence of acute GVHD and disease-associated mortality was difficult to evaluate in the event of early toxic death, patients who died within 30 days of transplantation (n = 53) also were excluded from the study. Among these 53 patients, 22 were evaluable for acute GVHD: grade 0 in 17 patients, grade 1-2 in 3 patients, and grade 3-4 in 2 patients. Two physicians (J.K. and T.I.) independently reviewed the quality of collected data, and 294 patients in total (158 males and 136 females), with a median age of 51 years (range, 18-79 years), were found to meet these criteria and included in the study: 163 patients from JSHCT, 82 patients from JMDP, and 49 patients from JCBBN. No overlapping cases were identified. Of these 294 patients, the effects of chronic GVHD, reported and graded according to using traditional criteria,[25] were considered evaluable for the 183 patients who survived at least 100 days after transplantation with complete information on the type and the day of occurrence of chronic GVHD.

End points

The primary end point of the study was the effect of acute GVHD on overall survival, defined as the period from the date of transplantation until the date of death from any cause or the last follow-up. The secondary end points of the study included the impact of acute GVHD on disease-associated and treatment-related mortality, and the impact of chronic GVHD on overall survival, disease-associated mortality, and treatment-related mortality. Reported causes of death were reviewed and categorized into disease-associated or treatment-associated deaths. Disease-associated deaths were defined as deaths from relapse or progression of ATL, whereas treatment-related deaths were defined as any death other than disease-associated deaths.

Statistical analysis

The probability of overall survival was estimated by the Kaplan-Meier method. Treatment-related and disease-associated mortality were estimated with the use of cumulative incidence curves to accommodate the following competing events[26]: disease-associated death for treatment-related mortality and treatment-related deaths for disease-associated mortality. Data on patients who were alive at the time of last follow-up were censored. Semi-landmark plots were used to illustrate the effects of GVHD on overall survival and cumulative incidence of disease-associated and treatment-related deaths. For patients with acute or chronic GVHD, the probability of overall survival and the cumulative incidences of disease-associated and treatment-related deaths were plotted as a function of time from the onset of acute or chronic GVHD. Day 24.5, the median day of onset for acute GVHD, was termed as the landmark day in patients without acute GVHD. In the case of patients without chronic GVHD, day 116, the median day of onset for chronic GVHD, was termed as the landmark day.

Univariate and multivariate Cox proportional hazards regression models were used to evaluate variables potentially affecting overall survival, whereas the Fine and Gray proportional subdistribution hazards models were used to evaluate variables potentially affecting disease-associated and treatment-related mortality.[27] In these regression models, the occurrence of acute and chronic GVHD was treated as a time-varying covariate.[28] In the analysis of acute GVHD, patients were assigned to the "no acute GVHD group" at the time of transplantation and then transferred to the "grade 1-2 acute GVHD group" or to the "grade 3-4 acute GVHD group" at the onset of the maximum grade of acute GVHD. In the analysis of chronic GVHD, patients were assigned to the "no chronic GVHD group" at the time of transplantation and then transferred to the "limited chronic GVHD group" or to the "extensive chronic GVHD group" at the onset of the maximum grade of chronic GVHD. The variables considered were the age group of the recipient ($\leq$ 50 years or > 50 years at transplantation), sex of the recipient (female or male), disease status before transplantation (complete remission, disease status other than complete remission, or unknown), intensity of conditioning regimen (myeloablative, reduced intensity, or unclassifiable), type of GVHD prophylaxis (cyclosporine-based, tacrolimus-based, or other), type of donor (HLA-matched related donor, HLA-mismatched related donor, unrelated donor for bone marrow, or unrelated cord blood), time from diagnosis to transplantation (within 6 months, > 6 months, or unknown), and year of transplantation (1995-2002 or 2003-2005). We classified the intensity of conditioning regimen as myeloablative or reduced intensity based on the working definition by Center for International Blood and Marrow Transplant Research if data on dosage of agents and total-body irradiation (TBI) used in the conditioning regimen were available.[29] For 110 patients for whom such information was not fully available, we used the information on conditioning intensity (myeloablative or reduced intensity) reported by treating clinicians. The cutoff points for year of transplantation were chosen such that we could make optimal use of the data with a proviso that the smaller group contained at least 30% of patients. In the analysis of the effect of chronic GVHD, the prior history of grade 2-4 acute GVHD also was added to the multivariate models. We also assessed the interaction between acute GVHD and the intensity of conditioning regimen in the multivariate models. Only factors with a P value of less than .10 in univariate analysis were included in the multivariate models. In addition, the heterogeneities of the effects of grade 1-2 or grade 3-4 acute GVHD on overall survival according to background transplant characteristics were evaluated by the forest plots stratified by variables included in the regression analyses. Furthermore, landmark analysis treating the development of acute GVHD as a time-fixed covariate was performed to confirm

Table 1. Characteristics of patients and transplants

Variable	No. of patients, n = 294 (%)
Age group at transplant, y	
≤ 30	7 (2)
> 30-40	30 (10)
> 40-50	109 (37)
> 50-60	123 (42)
> 60	25 (9)
Sex	
Male	158 (54)
Female	136 (46)
Disease status	
Complete remission	99 (34)
Not in complete remission	178 (61)
Unknown	17 (6)
Conditioning regimen	
Myeloablative	102 (34)
Reduced intensity	128 (44)
Unclassifiable	64 (22)
GVHD prophylaxis*	
Cyclosporine-based	195 (66)
Tacrolimus-based	94 (32)
Other	5 (2)
Source of stem cells	
Bone marrow	132 (45)
Peripheral blood	111 (38)
Bone marrow + peripheral blood	2 (1)
Cord blood	49 (17)
Type of donor†	
HLA-matched related	132 (45)
HLA-mismatched related	31 (11)
Unrelated, bone marrow	82 (28)
Unrelated, cord blood	49 (17)
Time from diagnosis to transplant	
≤ 6 mo	141 (48)
> 6 mo	141 (48)
Uncertain/missing	12 (4)
Year of transplant	
1995-1999	22 (7)
2000-2002	91 (31)
2003-2005	181 (62)
Follow-up of survivors	
Median time, mo (range)	42.8 (1.5-102.3)

Data are numbers (%) unless specified otherwise.

*Cyclosporine-based indicates cyclosporine with or without other agents; tacrolimus-based indicates tacrolimus with or without other agents.

†HLA compatibility was defined according to the results of serologic or low-resolution molecular typing for HLA-A, B, and DR antigens.

the results of analyses treating the occurrence of acute GVHD as a time-varying covariate; the landmark day was set at day 68 after transplantation, the date until when more than 95% of patients developed acute GVHD.

Results are expressed as hazard ratios (HRs) and their 95% confidence intervals (CI). All tests were 2-sided, and a P value of less than .05 was considered to indicate statistical significance. All statistical analyses were performed with STATA Version 11 software (StataCorp).

Results

Characteristics of patients and transplants

Characteristics of the patients and transplants are shown in Table 1. Most of the patients received transplants at the age of 41 to 60 years (median, 51 years). The disease status at transplan-

tation was mainly defined as other than complete remission. The intensity of conditioning regimen was classified as myeloablative in 102 (35%) patients and reduced intensity in 128 (44%) patients; the remaining 64 (22%) patients were reported to receive cyclophosphamide plus TBI in 16 patients; busulfan plus cyclophosphamide in 15 patients; busulfan plus melphalan in 1 patient; purine analog-containing regimen in 6 patients; and other TBI-based regimens in 26 patients, although the intensity of these regimens was considered unclassifiable because of lack of dosage information. Cyclosporine-based prophylaxis against GVHD was used in more than half of patients. Patients underwent transplantation using HLA-matched related donor in 132 patients (45%), HLA-mismatched related donor in 31 patients (11%), unrelated bone marrow donor in 82 patients (28%), and unrelated cord blood unit in 49 patients (17%). Half of the patients received transplants within 6 months of diagnosis. The median time of follow-up among the survivors was 42.8 months (range, 1.5-102.3 months).

Effects of acute GVHD on overall survival

The median onset day of acute GVHD of any grade after transplantation was 24.5 (range, 5-133). Acute GVHD of grades 1-4, 2-4, and 3-4 occurred in 202 patients (69%), 150 patients (51%), and 65 patients (22%), respectively. The effect of acute GVHD on overall survival was evaluated using semi-landmark plots with reference to the following 3 categories: no acute GVHD, grade 1-2 acute GVHD, and grade 3-4 acute GVHD (Figure 1A). The impact of grade 1-2 or grade 3-4 acute GVHD on overall survival also was evaluated by forest plots stratified by background characteristics of patients and transplants (Figure 2). These analyses revealed that development of grade 1-2 acute GVHD was consistently associated with higher overall survival compared with the absence of acute GVHD, whereas occurrence of grade 3-4 acute GVHD was consistently associated with lower overall survival, except that adverse impact of grade 3-4 acute GVHD was not observed in the subgroups of patients who received transplants from an HLA-matched related or HLA-mismatched related donor. Multivariate analysis treating an occurrence of acute GVHD as a time-dependent covariate also confirmed the positive impact of grade 1-2 acute GVHD (HR, 0.65; 95% CI, 0.45-0.93; P = .018) and the adverse impact of grade 3-4 acute GVHD on overall survival (HR, 1.64; 95% CI, 1.10-2.42; P = .014; Table 2). Patients who received reduced intensity conditioning and myeloablative conditioning had similar rates of overall survival by both univariate (HR of reduced intensity vs myeloablative transplant, 1.19; 95% CI, 0.85-1.68; P = .318) and multivariate analysis (HR, 0.95; 95% CI, 0.61-1.47; P = .814). There was no interaction effect between conditioning intensity and grade 1-2 (P = .704) or grade 3-4 acute GVHD (P = .891) on overall survival. The effect of each grade of acute GVHD on overall survival was additionally evaluated. It showed that only grade 2 acute GVHD was associated with superior overall survival, whereas only grade 4 acute GVHD was associated with inferior survival (supplemental Table 1, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). In the landmark analysis treating an occurrence of acute GVHD as a time-fix covariate, consistent results were obtained for patients who survived at least 68 days (landmark day), although the adverse impact of grade 3-4 acute GVHD on overall survival became no longer significant (supplemental Table 2).
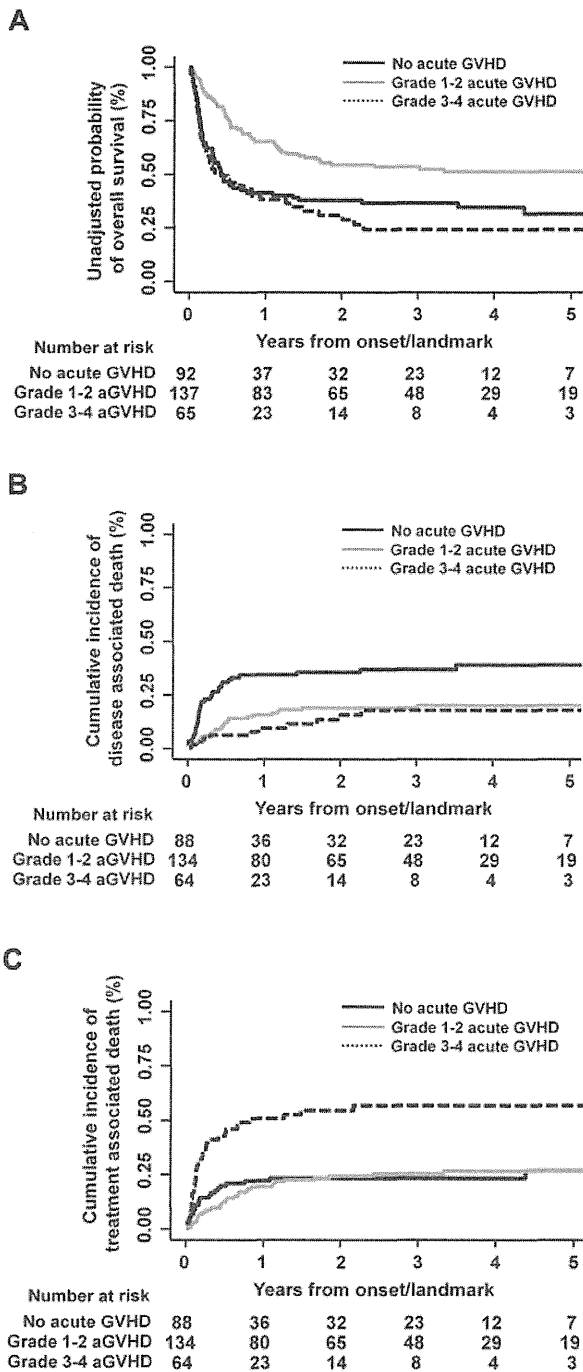
A

Number at risk						
No acute GVHD	92	37	32	23	12	7
Grade 1-2 aGVHD	137	83	65	48	29	19
Grade 3-4 aGVHD	65	23	14	8	4	3

B

Number at risk						
No acute GVHD	88	36	32	23	12	7
Grade 1-2 aGVHD	134	80	65	48	29	19
Grade 3-4 aGVHD	64	23	14	8	4	3

C

Number at risk						
No acute GVHD	88	36	32	23	12	7
Grade 1-2 aGVHD	134	80	65	48	29	19
Grade 3-4 aGVHD	64	23	14	8	4	3

Figure 1. Semi-landmark plots for effects of acute GVHD. Semi-landmark plots illustrating the effects of acute GVHD on overall survival (A), disease-associated mortality (B), and treatment-related mortality (C).

Effects of acute GVHD on disease-associated and treatment-related mortality

We next evaluated the effects of acute GVHD on disease-associated and treatment-related mortality (Figure 1B-C). Disease-associated mortality was defined as cumulative incidence of death directly attributable to relapse or progression of ATL, whereas treatment-related mortality was calculated as cumulative incidence of any death not included in disease-associated deaths. Multivariate analysis revealed that disease-associated mortality was lower in the presence of grade 1-2 and grade 3-4 acute GVHD compared with

the absence of acute GVHD (grade 1-2 acute GVHD: HR, 0.54; 95% CI, 0.32-0.92; $P = .023$ and grade 3-4 acute GVHD: HR, 0.44; 95% CI, 0.22-0.90; $P = .024$; Table 2), and each grade of acute GVHD showed consistent inverse association with disease-associated mortality (supplemental Table 1). Although the risk of treatment-related mortality was not higher in the presence of grade 1-2 acute GVHD, development of grade 3-4 acute GVHD was significantly associated with higher treatment-related mortality compared with the absence of acute GVHD (HR, 3.50; 95% CI, 2.01-6.11; $P < .001$; Table 2). Patients undergoing reduced intensity transplantation and those undergoing myeloablative transplantation had similar risks of disease-associated death (HR, 0.99; 95% CI, 0.46-2.13; $P = .975$) and treatment-related death (HR, 0.98; 95% CI, 0.60-1.59; $P = .928$) by multivariate analysis. There was no interaction effect between conditioning intensity and grade 1-2 or grade 3-4 acute GVHD on disease-associated mortality and treatment-related mortality. Of 95 patients who experienced treatment-related deaths, 27 patients succumbed to infectious complications: bacterial in 13 patients, viral in 7 patients (including 3 cases of cytomegalovirus disease), viral and bacterial in 1 patient, fungal in 5 patients, and no specific organism reported in 1 patient. The proportions of patients who died of infectious complication among those without acute GVHD (n = 92), those with grade 1-2 (n = 137), and those with grade 3-4 acute GVHD (n = 65) were 4%, 9%, and 17%, respectively (supplemental Table 3). By multivariate analysis, development of grade 3-4 acute GVHD was significantly associated with higher risk of death related to infection (HR, 4.74; 95% CI, 1.51-14.8; $P = .008$), whereas the adverse influence on the infection-related deaths was less evident in the presence of grade 1-2 acute GVHD (HR, 2.17; 95% CI, 0.72-6.56; $P = .169$).

Effects of chronic GVHD on overall survival and mortality

Chronic GVHD was evaluated in 183 patients who survived at least 100 days after transplantation. The median day of chronic GVHD occurrence after transplantation was 116 (range, 100-146 days). Limited and extensive chronic GVHD occurred in 29 (16%) and 63 patients (34%), respectively. Semi-landmark plots were constructed to illustrate the effects of chronic GVHD on overall survival, disease-associated mortality, and treatment-related mortality with reference to the following subgroups: no chronic GVHD, limited chronic GVHD, and extensive chronic GVHD (Figure 3). In multivariate analysis treating an occurrence of chronic GVHD as a time-dependent covariate, neither overall survival nor disease-associated mortality was significantly associated with severity of chronic GVHD, whereas treatment-related mortality was higher in the presence of extensive chronic GVHD (HR, 2.75; 95% CI, 1.34-5.63; $P = .006$) compared with the absence of chronic GVHD (Table 3). The proportions of patients who died of infectious complication among those without chronic GVHD (n = 91), those with limited chronic GVHD (n = 29), and those with extensive chronic GVHD (n = 63) were 7%, 10%, and 8%, respectively. In multivariate analysis, no statistically significant association was found between infection-related death and the occurrence of either limited ($P = .289$) or extensive GVHD ($P = .836$).

Discussion

To our knowledge, this is the largest retrospective study to analyze the impact of acute and chronic GVHD on clinical
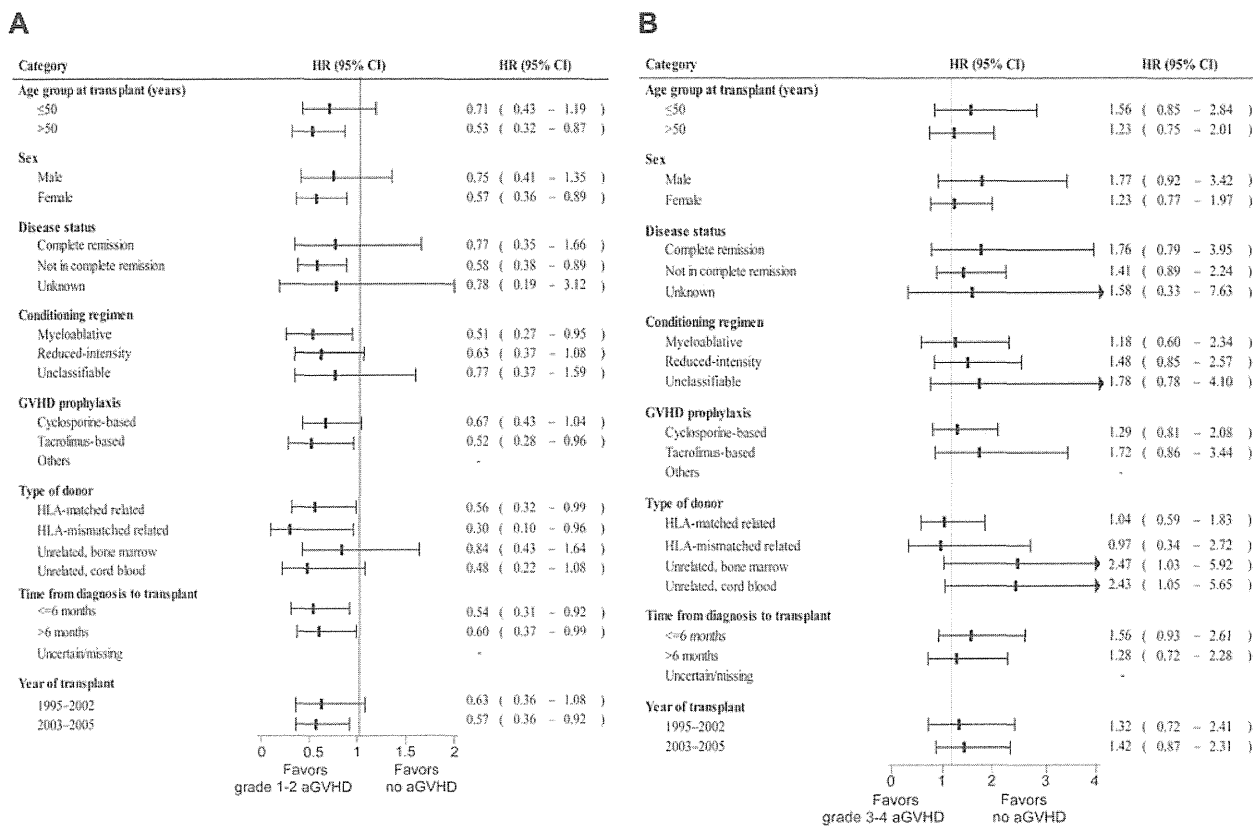
Figure 2. Impact of the grade of acute GVHD on overall survival in each stratified category. Effects of grade 1-2 (A) and grade 3-4 acute GVHD (B) on overall survival are shown as forest plots. Square boxes on lines indicate hazard ratios compared with "no acute GVHD group," and horizontal lines represent the corresponding 95% CI. Abbreviations used are the same as described in the footnotes to Tables 1 and 2.

outcomes including overall survival, disease-associated mortality, and treatment-related mortality after allogeneic HCT for ATL. In the present study, the occurrence of both grade 1-2 and grade 3-4 acute GVHD was associated with lower disease-associated mortality compared with the absence of acute GVHD. However, positive effect of GVHD on reduced disease-associated mortality was counterbalanced by increased treatment-

related mortality among patients who developed severe acute GVHD, and an overall beneficial effect on survival was observed only with the development of mild-to-moderate acute GVHD. In contrast to acute GVHD, no beneficial effect was observed in association with the development of chronic GVHD, although the point estimate of the HR comparing limited chronic GVHD versus the absence of chronic GVHD

Table 2. Effect of acute GVHD on overall survival, disease-associated mortality, and treatment-related mortality after allogeneic hematopoietic cell transplantation for adult T-cell leukemia

Outcome	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P	HR (95% CI)	P
Overall survival*				
Grade 1 or 2 acute GVHD vs no acute GVHD	0.60 (0.42-0.85)	.004	0.65 (0.45-0.93)	.018
Grade 3 or 4 acute GVHD vs no acute GVHD	1.38 (0.94-2.01)	.099	1.64 (1.10-2.42)	.014
Disease-associated mortality†				
Grade 1 or 2 acute GVHD vs no acute GVHD	0.47 (0.28-0.79)	.005	0.54 (0.32-0.92)	.023
Grade 3 or 4 acute GVHD vs no acute GVHD	0.41 (0.21-0.81)	.010	0.44 (0.22-0.90)	.024
Treatment-related mortality‡				
Grade 1 or 2 acute GVHD vs no acute GVHD	1.13 (0.67-1.89)	.649	1.22 (0.72-2.07)	.461
Grade 3 or 4 acute GVHD vs no acute GVHD	3.34 (1.94-5.74)	< .001	3.50 (2.01-6.11)	< .001

*Other significant variables were sex of recipient, female (reference, 1.00) and male (HR, 1.70; 95% CI, 1.24-2.32; P = .001); achievement of complete remission, complete remission (reference, 1.00), status other than complete remission (HR, 2.05; 95% CI, 1.44-2.92; P < .001), and status not known (HR, 2.21; 95% CI, 1.15-4.22; P = .017); type of donor, HLA-matched related donor (reference, 1.00), HLA-mismatched related donor (HR, 1.71; 95% CI, 1.04-2.84; P = .036), unrelated donor of bone marrow (HR, 1.39; 95% CI, 0.94-2.06; P = .096), and unrelated cord blood (HR, 1.86; 95% CI, 1.22-2.83; P = .004).

†Other significant variables were achievement of complete remission, complete remission (reference, 1.00), status other than complete remission (HR, 2.98; 95% CI, 1.62-5.47; P < .001), and status not known (HR, 0.96; 95% CI, 0.21-4.49; P = .963); type of donor, HLA-matched related donor (reference, 1.00), HLA-mismatched related donor (HR, 2.14; 95% CI, 1.00-4.55; P = .049), unrelated donor of bone marrow (HR, 1.45; 95% CI, 0.81-2.61; P = .214), and unrelated cord blood (HR, 1.25; 95% CI, 0.63-2.49; P = .517).

‡Another significant variable was achievement of complete remission, complete remission (reference, 1.00), status other than complete remission (HR, 1.17; 95% CI, 0.74-1.84; P = .498) and status not known (HR, 2.31; 95% CI, 1.04-5.15; P = .040).

A

Number at risk	Years from onset/landmark				
	0	1	2	3	4	5
No chronic GVHD	88	53	47	36	21	11
Limited chronicGVHD	29	25	20	11	6	5
Extensive chronic GVHD	63	39	28	19	11	9

B

Number at risk	Years from onset/landmark				
No chronic GVHD	85	50	47	36	21	11
Limited chronicGVHD	29	25	20	11	6	5
Extensive chronic GVHD	62	38	28	19	11	9

C

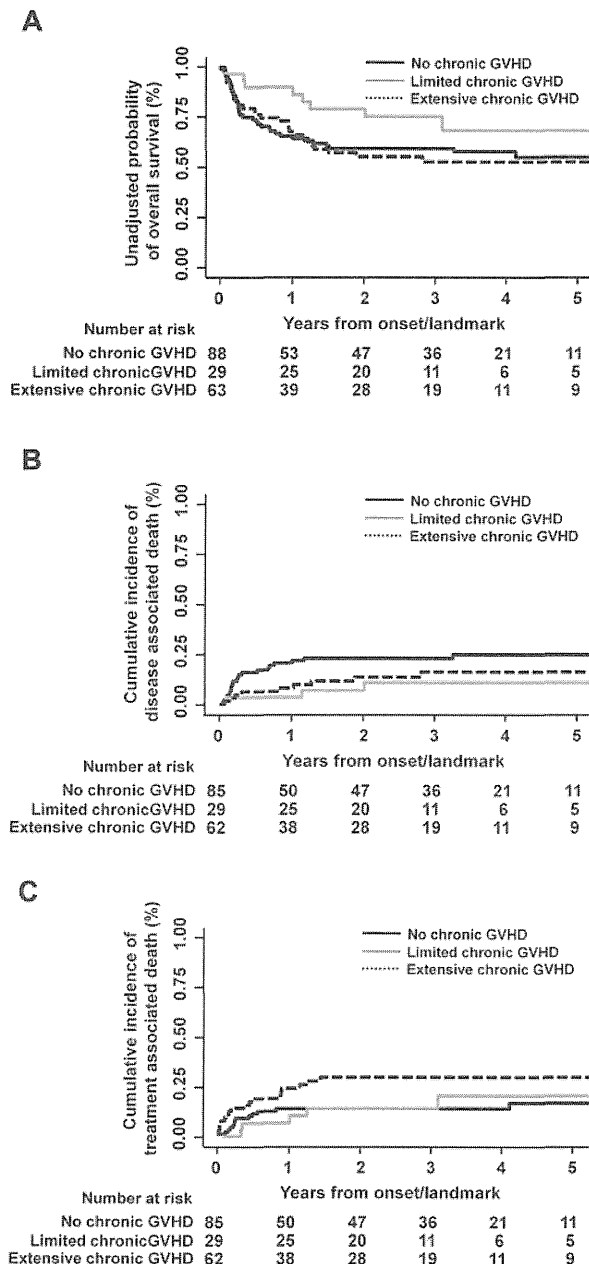| Number at risk	Years from onset/landmark				
No chronic GVHD	85	50	47	36	21	11
Limited chronicGVHD	29	25	20	11	6	5
Extensive chronic GVHD	62	38	28	19	11	9

Figure 3. Semi-landmark plots for impact of chronic GVHD. Semi-landmark plots illustrating impact of chronic GVHD on overall survival (A), disease-associated mortality (B), and treatment-related mortality (C).

suggested the trend toward a reduced risk of disease-associated deaths in the limited chronic GVHD group.

Our present findings are in contrast to the previous reports showing the beneficial effects of chronic GVHD rather than acute GVHD on the prevention of disease recurrence after allogeneic HCT. It is less likely that the particular characteristics of chronic GVHD in patients with ATL biased the results, because the incidence rate and median onset day of chronic GVHD in our cohort were similar to those reported in previous studies evaluating the incidence of chronic GVHD among Japanese patients, most of whom had received allogeneic HCT for myeloid neoplasms or acute lymphoblastic leukemia.[30-32] Conceivably, the rapid tempo of disease recurrence of ATL might be such that chronic GVHD is less potent in terms of harnessing clinically relevant graft-versus-

leukemia responses compared with acute GVHD. However, the results of our analysis regarding the effect of chronic GVHD should be interpreted with caution because the number of patients evaluable for chronic GVHD was relatively small in our study for providing sufficient statistical power. The effect of chronic GVHD on outcomes after HCT for ATL should be further explored in a larger cohort.

The occurrence of GVHD has been shown to exert a potent graft-versus-leukemia effect in terms of reducing relapse incidence in acute leukemia or chronic myeloid leukemia.[33,34] In contrast, multiple studies have documented a correlation between GVHD in its acute or chronic form and treatment-related mortality. In a study of patients undergoing HLA-identical sibling HCT for chronic myeloid leukemia, the overall beneficial effect on long-term survival was demonstrated only in a group of patients who developed grade 1 acute GVHD or limited chronic GVHD.[33] In another study of HLA-identical sibling HCT for leukemia using cyclosporine and methotrexate as GVHD prophylaxis, a benefit of mild GVHD was only seen in high-risk patients but not in standard-risk patients. Therefore, the therapeutic window between decreased relapse incidence and increased transplant-related mortality in association with the development of GVHD has been considered to be very narrow.[34]

With regard to the effectiveness of allogeneic HCT for ATL, it is also of note here that posttransplant eradication of ATL cells can be achieved without the use of high-dose chemoradiotherapy: patients who received a transplant with reduced intensity conditioning had survival outcomes similar to those who received a transplant with myeloablative conditioning in our study. Intriguingly, several small cohort studies exhibited that abrupt discontinuation of immunosuppressive agents resulted in disappearance or reduction in the tumor burden in allografted patients with ATL. In some cases, remission of ATL was observed along with the development of GVHD.[19,20,22] Taken together with the findings of this study, it is suggested that ATL is particularly susceptible to immune modulation following allogeneic HCT. To clarify the presence of such "graft-versus-ATL" effect, further investigations are needed to assess the efficacy of donor lymphocyte infusion or withdrawal of immunosuppressive agents on relapse after transplantation.

Of the HTLV-I gene products, Tax is a dominant target of HTLV-I–specific cytotoxic T lymphocytes. The vigorous Tax-specific cytotoxic T-cell responses were demonstrated in recipients who obtained complete remission after allogeneic HCT for ATL, suggesting that "graft-versus-HTLV-I" responses might contribute to the eradication of ATL cells.[35,36] However, Tax is generally undetectable or present in very low levels in primary ATL cells.[37,38] In addition, small amounts of HTLV-I provirus can be detected in peripheral blood of recipients who attained long-term remission of ATL, even after HCT from HTLV-I–negative donors.[39,40] These findings suggest that "graft-versus-ATL" effect can be harnessed without complete elimination of HTLV-I. It is also important to note that allogeneic HCT is emerging as an effective treatment option for other mature T-cell neoplasms not related to HTLV-I, such as mycosis fungoides/Sézary syndrome and various types of aggressive peripheral T-cell lymphomas.[41,42] These observations raised the possibility that the common targets for alloimmune responses might exist across a spectrum of malignant T-cell neoplasms, including ATL. The minor histocompatibility antigens or tumor-specific antigens can be other targets of alloimmune anti-ATL effect.[43-45] Therefore, the elucidation of the mechanism underlying an immunologic eradication of primary ATL cells may

Table 3. Effect of chronic GVHD on overall survival, disease-associated mortality, and treatment-related mortality after allogeneic hematopoietic cell transplantation for adult T-cell leukemia

Outcome	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P	HR (95% CI)	P
Overall survival*				
Limited chronic GVHD vs no chronic GVHD	0.71 (0.34-1.47)	.353	0.72 (0.35-1.50)	.385
Extensive chronic GVHD vs no chronic GVHD	1.45 (0.90-2.35)	.131	1.40 (0.86-2.30)	.176
Disease-associated mortality†				
Limited chronic GVHD vs no chronic GVHD	0.45 (0.14-1.46)	.183	0.45 (0.14-1.44)	.178
Extensive chronic GVHD vs no chronic GVHD	0.81 (0.39-1.67)	.563	0.80 (0.39-1.64)	.536
Treatment-related mortality‡				
Limited chronic GVHD vs no chronic GVHD	1.59 (0.64-3.95)	.316	1.56 (0.63-3.87)	.342
Extensive chronic GVHD vs no chronic GVHD	2.85 (1.41-5.77)	.004	2.75 (1.34-5.63)	.006

*There was no significant variable.
†There was no significant variable.
‡There was no other significant variable.

lead to a new strategy for improving outcomes of allogeneic HCT not only for ATL but also for other intractable T-cell neoplasms.

This study has several limitations. First, acute GVHD might be intentionally induced for some patients considered at high risk of relapse by treating clinicians. Second, the information on the day when each grade of GVHD occurred was not available. Therefore, we treated the development of acute and chronic GVHD in their worst severity as a time-varying covariate. To validate the results, we also performed the landmark analysis and obtained consistent results. Third, the relatively small number of patients with chronic GVHD might mask or bias the effect of chronic GVHD on outcomes. Last, the effect of multiple testing should be taken into account for the interpretation of the secondary end points.

In conclusion, the development of acute GVHD was associated with lower disease-associated mortality after allogeneic HCT for ATL compared with the absence of acute GVHD. However, improved survival can be expected only among a group of patients who developed mild-to-moderate acute GVHD because those who developed severe acute GVHD were at high risk of treatment-related mortality. New strategies that enhance the allogeneic anti-ATL effect without exacerbating GVHD are required to improve the outcomes of patients undergoing allogeneic HCT for ATL.

Acknowledgments

Authorship

Contribution: T.I. and T.U. designed the research and organized the project; M. Hishizawa, J.K., T.I., and T.U. reviewed and analyzed data and wrote the paper; J.K., T.I., and K.M. performed statistical analysis; Y.A., R.S., and H.S. collected data from JSHCT; T.K. and Y. Morishima collected data from JMDP; T.N.-I., and S. Kato collected data from JCBBN; and A.U., S.T., T.E., Y. Moriuchi, R.T., F.K., Y. Miyazaki, M.M., K.N., M. Hara, M.T., S. Kai, and J.O. interpreted data and reviewed and approved the final manuscript.

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A list of other members who contributed data on allogeneic HSCT for ATL to JSHCT, JMDP, and JCBBN appears in the online supplemental Appendix.

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Efficacy and safety of human adipose tissue-derived mesenchymal stem cells for supporting hematopoiesis

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Abstract We have demonstrated that adipose tissue-derived mesenchymal stem cells (ADSCs) from mice are capable of reconstituting the hematopoietic microenvironment, and facilitate hematopoiesis more effectively than bone marrow-derived mesenchymal stem cells (BMSCs) in mouse. The ready accessibility of fat tissue rich in MSCs and the higher hematopoiesis-supporting capacities of ADSCs suggest that ADSCs might represent a new therapeutic modality for the regeneration of impaired hematopoiesis. As a further step towards their use in clinical practice, we established human BMSCs and ADSCs from healthy volunteers of similar age, and compared their proliferation capacities, hematopoiesis-supporting properties, and safety. In vitro cell proliferation studies revealed that ADSCs have a higher population doubling number than BMSCs. In vitro co-culture assays showed that ADSCs not only support human CD34$^+$ peripheral blood stem cells (PBSCs), but also yield significantly more non-adherent hematic cells than BMSCs. In vitro progenitor assays revealed that ADSCs promote a higher frequency of early progenitors than do BMSCs. Interestingly, BM cellularity in irradiated mice that had received ADSCs tended to be higher than that of mice treated with BMSCs. When MSCs were injected into the BM cavity of tibiae, we observed no evidence of MSC-induced toxicity either during or after treatment. In addition, no microscopic abnormalities were observed in the bone marrow and major organs.

Introduction

Hematopoiesis is a dynamic process that involves self renewal of hematopoietic stem cells in the bone marrow, generation of lineage-committed cells, and mobilization of mature cells into the bloodstream. Mesenchymal stem cells (MSCs) present in bone marrow (BM) are thought to give rise to cells that constitute the hematopoietic microenvironment. MSCs produce a number of cytokines and extracellular matrix proteins and express cell adhesion molecules, all of which are involved in the regulation of hematopoiesis [1].

The hematopoietic microenvironment can be damaged by various pathophysiological mechanisms such as chemotherapy, irradiation, aging and malignant disease [2, 3]. Not only intensive chemotherapy but also chemotherapeutic drugs alone disrupt the hematopoietic microenvironment [4, 5]. In the elderly, bone marrow structures tend to be replaced with adipocytes that can be negative regulators of the hematopoietic microenvironment [6].

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Long-lasting damage to the hematopoietic microenvironment impairs hematopoiesis, causing infection, bleeding, anemia and subsequent mortality.

The emerging field of regenerative medicine seeks to repair or restore lost or damaged tissue function due to the effects of injury, disease, and aging. Compelling studies showed that BM-derived mesenchymal stem cells (BMSCs), when directly injected into the BM, could reconstitute the hematopoietic microenvironment [7, 8]. These facts clearly suggest that BMSCs can be a new modality for regeneration of the hematopoietic microenvironment. However, there are several drawbacks in the use of BMSCs for clinical application. Even though it is desirable to establish MSCs from the patient to whom they will be administered due to the possibility that an immune response and subsequent adverse effects could be provoked by administration of allogeneic MSCs [9], BMSCs are only available in a limited number. To make matters worse, the number, differentiation potential, and maximal life span of BMSCs decline with increasing age [10, 11].

The discoveries that a large number of nonadipocyte stem cells exist in fat tissue (adipose tissue-derived MSCs; ADSCs) and that these cells can be rapidly expanded ex vivo suggested that ADSCs might be useful for clinical applications [12]. We recently reported that ADSCs are a better alternative to BMSCs for reconstitution of the hematopoietic microenvironment in a mouse model [13]. Since then, we have maintained an ongoing commitment to explore the potential of ADSCs for clinical applications. In this study, we provide evidence that human ADSCs support hematopoiesis better than human BMSCs.

Materials and methods

Animal studies

The animal experiments were approved by the institutional ethics committee for Laboratory Animal Research, Nagoya University School of Medicine, and were performed according to the guidelines of the institute.

Reagents and cells

RPMI 1640, heat-inactivated fetal bovine and horse serum, and α-minimal essential medium were purchased from Gibco-BRL (Carlsbad, CA, USA). Human BMSCs and ADSCs were established from healthy volunteers of similar age (20–30 years). Briefly, bone marrow cells and fat tissues were obtained with informed consent from four and five individuals, respectively, and were then processed as described elsewhere [14]. Before experimental use, we confirmed that the MSCs possessed the ability to differentiate into adipocytes and osteoblasts. Cultures between passages 4–8 were used. CD34$^+$ hematopoietic stem cells were mobilized by G-CSF into the periphery, collected and frozen at -130 °C until use.

In vitro cell proliferation studies

Human BMSCs and ADSCs were plated (5×10^3 cells/well, three independent determinations per MSC) onto 24-well plates. After 72-h incubation, the cells were trypsinized and viable cells were counted using trypan blue exclusion.

Co-culture of CD34$^+$ progenitor cells with BMSCs or ADSCs

1×10^5 human CD34$^+$ PBSCs suspended in long-term culture medium were applied (1×10^5 cells in 2 ml) to feeder layers comprising human BMSCs or human ADSCs, as described previously [13]. The co-cultures were incubated for 4 weeks with replenishment of the culture medium twice per week. Non-adherent viable cells were counted at the indicated time points and were analyzed by FACS at the end of incubation as described elsewhere [13]. Co-culture experiments were repeated three times.

In vitro progenitor assays

Effects of human MSCs on progenitor cells were analyzed using a colony-forming cell assay. The following human cells (5×10^2 each) were plated in 0.5 mL of methylcellulose media (Stemcell Technologies, Vancouver, Canada): BMSCs, ADSCs, CD34$^+$ PBSCs, PBSCs plus BMSCs and PBSCs plus ADSCs. Colonies of >50 cells were scored after 8-days of incubation. Experiments were repeated three times.

Intra-bone marrow transplantation

Human BMSCs, human ADSCs (1×10^5 cells each in 10 µl of RPMI 1640), or 10 µl of RPMI 1640 were injected into the right tibiae of irradiated (3.0 Gy) 6- to 8-week-old NOD/SCID mice (4–5 mice per subgroup, Chubu Kagaku Shizai, Nagoya, Japan) using a Hamilton syringe. All mice were killed 5 weeks after injection, and the tibiae and major organs were excised for histological evaluation. For quantitative analysis of BM cellularity, 4 fields were randomly selected and the number of nucleated cells was scored under a microscope.

Statistical analysis

Statistical significance of group differences was evaluated using Student's *t* test and Excel software (Microsoft, Redmond, WA, USA).

Results

Human ADSCs can be expanded faster than human BMSCs

An in vitro proliferation assay showed that the number of BMSCs increased 1.16- to 2.32-fold above the input cell number (5000 cells/well) after 72-h incubation. In contrast, the fold increase in the number of ADSCs (3.5–4.0) was significantly higher (Fig. 1a).

Human ADSCs support human CD34$^+$ PBSCs to a greater degree than human BMSCs

To analyze the ability of human ADSCs to induce granulocyte differentiation of human CD34$^+$ PBSCs, co-culture assays were performed. Human ADSCs yielded significantly more non-adherent cells from human CD34$^+$ PBSCs than human BMSCs (Fig. 1b, upper panel). As noted previously [13], round-shaped hematic cells grew in clusters, suspended in the culture supernatant or loosely attached to supportive stroma (Fig. 1b, lower right panel). FACS analysis of non-adherent cells showed that these cells differentiated into CD33$^+$ granulocytes derived from human CD34$^+$ PBSCs (Fig. 1b, lower right panel). These results suggest that human ADSCs significantly enhance proliferation of myeloid cells from human CD34$^+$ PBSCs compared with human BMSCs.

In vitro progenitor assays revealed that human ADSCs promoted a higher frequency of early progenitors than human BMSCs (Fig. 1c), whereas neither human BMSCs nor human ADSCs alone generated colonies (not shown). The major lineages of the colonies were colony-forming unit (CFU) granulocytes and CFU granulocyte-macrophages. Few erythrocyte colonies were observed. However, there was no significant difference in the percentage of CFU granulocytes or CFU granulocyte-macrophages within CD34$^+$ PBSCs in the presence or absence of BMSCs or ADSCs (data not shown). Representative results from three independent experiments are shown.

Safety of human ADSCs in vivo

To ensure the safety of human ADSCs in vivo, we injected human BMSCs or ADSCs (1 × 10^5 cells each) into the right tibiae of irradiated 6–8 weeks old NOD/SCID mice (one mouse per each individual MSC). We observed no evidence of MSC-induced toxicity (e.g., body weight loss or death) either during or after treatment. Histological evaluation 5 weeks after injection showed neither gross morphological nor microscopic change of heart, lung, liver, kidney or spleen (Fig. 2a). Similarly, no microscopic abnormalities such as fatty change or fibrosis were observed in the bone marrow of mice that received MSCs (Fig. 2b). Interestingly, BM cellularity in mice that had received human ADSCs was significantly higher than that of mice that had received human BMSCs (Fig. 2b).

Discussion

We have recently demonstrated that ADSCs from mice can facilitate hematopoiesis more effectively than BMSCs [13]. These data suggested that ADSCs possess clinical potential to facilitate hematopoiesis. However, human cells and mouse cells do not always behave similarly. To determine the hematopoiesis-supporting properties of human ADSCs, we established BMSCs and ADSCs from a number of individuals of similar age, because the number, differentiation potential, and maximal life span of BMSCs decline with increasing age [11] while ADSCs are abundant even in the elderly [15]. Although we were not able to establish BMSCs and ADSCs from the same individual, in vitro coculture and progenitor assays clearly showed that human ADSCs generated significantly more granulocytes and progenitor cells from human hematopoietic stem cells (HSCs) than human BMSCs (Fig. 1b, c). Intra-bone marrow transplantation experiments revealed that BM cellularity in mice that had received human ADSCs was significantly higher than that of mice that had received human BMSCs (Fig. 2b). These data clearly suggest that human ADSCs are superior to human BMSCs in terms of hematopoiesis-supporting properties. Bone marrow failure is a heterogeneous disease that is caused by various pathophysiological mechanisms including immune destruction of hematopoiesis, quantitative and qualitative defects in hematopoietic stem cells in addition to perturbation of the hematopoietic microenvironment [2, 16]. ADSCs are useful, not only for supporting hematopoiesis, but also for modulation of immunoreactions [17, 18]. In addition, ADSCs freshly isolated from fat tissue (adipose-derived stem and regenerative cells) using the Celution® system (Cytori Therapeutics, Inc. http://www.cytori.com/Home.aspx) may contain hematopoietic stem and progenitor cells [19]. Again, our data and these facts suggest that fat tissue is a good source of cells that can be used for therapy to reconstitute impaired hematopoiesis.

Analysis of the proliferation capacities showed that ADSCs possessed higher population doubling numbers than BMSCs, which is consistent with a previous report [20]. A prospective randomized study using MSCs as first-line therapy for graft failure after hematopoietic stem cell transplantation (HSCT) showed that two out of six patients with poor hematopoietic recovery after HSCT responded to the infusion of BMSCs (1 × 10^6/kg) and their blood cell counts increased [21]. The authors speculated that
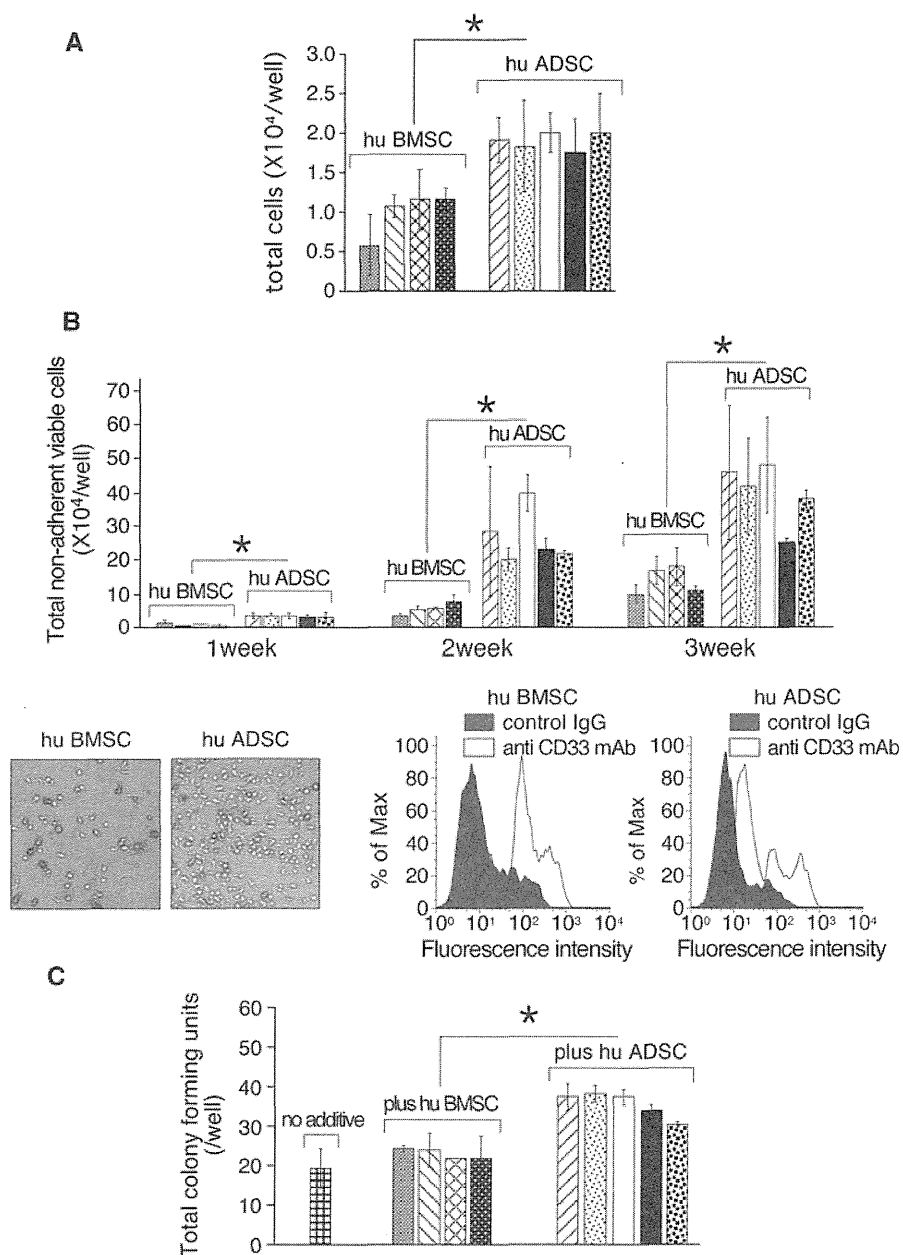
Fig. 1 In vitro comparison of human BMSCs and ADSCs. a In vitro proliferation of human BMSCs and ADSCs. Human BMSCs and human ADSCs were plated (5×10^3 cells/well, three independent determinations per MSC) onto 24-well plates. After 72-h incubation the cells were trypsinized and viable cells were counted using trypan blue exclusion. *Each bar* represents cells from one individual. The results are presented as the mean ± SD. The *asterisk* denotes statistical significance (*$P < 0.05$). b Co-culture of CD34$^+$ PBMCs with human MSCs. BMSCs and ADSCs were established from four and five individuals, respectively. Cell layers of BMSCs and ADSCs were established on 0.5 % gelatin pre-coated 24-well plates (80 % confluent). CD34$^+$ PBSCs were applied onto the stromal layers (4 wells/each MSC). The cocultures were incubated for 3 weeks with replenishment of the culture medium twice a week. Non-adherent viable cells were counted at the indicated time points (*upper panel*), photographed (*lower left panel*, representative photographs), and analyzed by FACS at the end of the incubation (*lower right panel*, representative results). Each point represents the mean (±SD) of four replicates. The *asterisk* denotes statistical significance (*$P < 0.05$). c In vitro progenitor assays using human MSCs. Human CD34$^+$ peripheral blood stem cells (PBSCs: 500 cells, no additive), PBSCs plus BMSCs (500 cells each) or PBSCs plus ADSCs (500 cells each) were plated in 0.5 mL of methylcellulose media containing human recombinant IL-3, SCF, and Epo (3 wells/each MSC). The plates were incubated for 8 days following which progenitors were scored. The results represent the mean (±SD) of three replicates. The *asterisk* denotes statistical significance (*$P < 0.05$)