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Continuing increased risk of oral/esophageal cancer after allogeneic hematopoietic stem cell transplantation in adults in association with chronic graft-versus-host disease

Y. Atsuta[1,*], R. Suzuki[1], T. Yamashita[2], T. Fukuda[2], K. Miyamura[3], S. Taniguchi[4], H. Iida[5], T. Uchida[6], K. Ikegame[7], S. Takahashi[8], K. Kato[9], K. Kawa[10], T. Nagamura-Inoue[11], Y. Morishima[12], H. Sakamaki[13] & Y. Kodera[14], for the Japan Society for Hematopoietic Cell Transplantation

[1]*Department of HSCT Data Management and Biostatistics, Nagoya University Graduate School of Medicine, Nagoya;* [2]*Hematopoietic Stem Cell Transplantation Unit, National Cancer Center Hospital, Tokyo;* [3]*BMT Center, Japanese Red Cross Nagoya First Hospital, Nagoya;* [4]*Department of Hematology, Toranomon Hospital, Tokyo;* [5]*Department of Hematology, Meitetsu Hospital, Nagoya;* [6]*Department of Hematology and Oncology, Nagoya Daini Red Cross Hospital, Nagoya;* [7]*Division of Hematology, Department of Internal Medicine, Hyogo College of Medicine, Nishinomiya;* [8]*Department of Molecular Therapy, The Institute of Medical Science, The University of Tokyo, Tokyo;* [9]*Department of Pediatrics, Japanese Red Cross Nagoya First Hospital, Nagoya;* [10]*Osaka Medical Center and Research Institute for Maternal and Child Health, Izumi;* [11]*Department of Cell Processing and Transfusion, Research Hospital, The Institute of Medical Science, The University of Tokyo, and Tokyo Cord Blood Bank, Tokyo;* [12]*Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya;* [13]*Division of Hematology, Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital, Tokyo;* [14]*Department of Promotion for Blood and Marrow Transplantation, Aichi Medical University, School of Medicine, Nagakute, Japan*

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Background: The number of long-term survivors after hematopoietic stem cell transplantation (HSCT) showed steady increase in the past two decades. Second malignancies after HSCT are a devastating late complication. We analyzed the incidence of, risk compared with that in the general population, and risk factors for secondary solid cancers.
Patients and methods: Patients were 17 545 adult recipients of a first allogeneic stem cell transplantation between 1990 and 2007 in Japan. Risks of developing secondary solid tumors were compared with general population by using standard incidence ratios (SIRs).
Results: Two-hundred sixty-nine secondary solid cancers were identified. The cumulative incidence was 0.7% [95% confidence interval (CI), 0.6%–0.9%] at 5 years and 1.7% (95% CI, 1.4%–1.9%) at 10 years after transplant. The risk was significantly higher than that in the general population (SIR = 1.8, 95% CI, 1.5–2.0). Risk was higher for oral cancer (SIR = 15.7, 95% CI, 12.1–20.1), esophageal cancer (SIR = 8.5, 95% CI, 6.1–11.5), colon cancer (SIR = 1.9, 95% CI, 1.2–2.7), skin cancer (SIR = 7.2, 95% CI, 3.9–12.4), and brain/nervous system cancer (SIR = 4.1, 95% CI, 1.6–8.4). The risk of developing oral, esophageal, or skin cancer was higher at all times after 1-year post-transplant. Extensive-type chronic graft-versus-host disease (GVHD) was a significant risk factor for the development of all solid tumors (RR = 1.8, $P < 0.001$), as well as for oral (RR = 2.9, $P < 0.001$) and esophageal (RR = 5.3, $P < 0.001$) cancers. Limited-type chronic GVHD was an independent risk factor for skin cancers (RR = 5.8, $P = 0.016$).
Conclusion: Recipients of allogeneic HSCT had a significantly higher ~2-fold risk of developing secondary solid cancers than the general population. Lifelong screening for high-risk organ sites, especially oral or esophageal cancers, is important for recipients with active, or a history of, chronic GVHD.
Key words: secondary solid cancers, late effect, hematopoietic stem cell transplantation

introduction

Hematopoietic stem cell transplantation (HSCT) is a curative treatment of choice for malignant and non-malignant hematological disorders [1]. The annual number of allogeneic HSCT has increased steadily over the past three decades worldwide [2–6]. Progress in transplant procedures in addition to this steady increase in the number of HSCT procedures worldwide has contributed to an increase in the number of long-term survivors.

Secondary malignancies, including new solid cancers, are an important cause of late mortality. Several studies have reported that survivors of HSCT have a 2–3-fold increased risk of

**Correspondence to*: Dr Yoshiko Atsuta, Department of Hematopoietic Stem Cell Transplantation Data Management and Biostatistics, Nagoya University Graduate School of Medicine, 1-1-20 Daiko-Minami, Higashi-ku, Nagoya 461-0047, Japan. Tel: +81-52-719-1973; Fax: +81-52-719-1973; E-mail: y-atsuta@med.nagoya-u.ac.jp

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developing new solid cancers compared with an age-, sex-, region-, and calendar-year-adjusted population and the risk among long-term survivors ranges from 1% to 6% at 10 years after transplantation [7–14]. Identified risk factors include exposure to radiation as a part of the conditioning regimen and chronic graft-versus-host disease (GVHD), and the latter has been shown to be strongly correlated with the development of squamous cell carcinoma [8, 10, 12, 15–17]. However, a recent long-term follow-up analysis of patients who were transplanted after myeloablative doses of busulfan and cyclophosphamide without total body irradiation (TBI) found a similar increased incidence of 0.6% at 5 years and 1.2% at 10 years after transplantation [13]. We conducted a nationwide, retrospective cohort study with a large and different cohort from those used in previous reports from North America and Europe, to determine the incidence and risks of developing secondary solid cancers.

methods

data source and collection of data

The recipient clinical data were collected by the Japan Society for Hematopoietic Cell Transplantation (JSHCT) using the Transplant Registry Unified Management Program, as described previously [18]. The JSHCT collect recipients' baseline, disease, transplant, and transplant outcome information who received HSCT in the previous year. Patient information regarding survival, disease status, and long-term complications including chronic GVHD and second malignancies are renewed annually. This study was approved by the data management committee of the JSHCT, as well as the institutional review board of Nagoya University Graduate School of Medicine.

patients

Adult patients (at least 16 years of age) who received a first HSCT between 1990 and 2007 were considered as subjects for the present study. Those who were inherently susceptible to developing cancer [Fanconi anemia ($N = 3$) and congenital immunodeficiency ($N = 12$)] were excluded. Three-hundred five recipients (1.7%) were excluded because of insufficient follow-up data. The study included 17 545 recipients; 5358 recipients of related bone marrow, 3587 recipients of related peripheral blood stem cells (including 134 bone marrow and peripheral blood stem cells combined), 6508 recipients of unrelated bone marrow, and 2092 recipients of unrelated cord blood.

statistical analysis

Standard incidence ratios (SIRs) were calculated to determine whether the number of recipients in the present cohort who developed secondary solid tumor after receiving a HSCT was different than that in the general population (supplementary method, available at *Annals of Oncology* online). Cumulative incidences of solid cancer or GVHD were estimated by taking into account the competing risk of death among patients who did not develop a second malignancy or GVHD [19]. The influence of potential risk factors was estimated by using the Cox proportional hazard model [20]. A stepwise multivariate approach was used to identify the most important predictor with respect to the development of secondary solid cancers. The variables considered were age at transplant, patient sex, donor-type (related versus unrelated), graft source, TBI as part of the conditioning regimen, reduced-intensity conditioning, grade 2–4 acute GVHD, and chronic GVHD. The model was stratified into four categories according to the primary disease; acute myeloid leukemia, acute lymphoblastic leukemia, chronic myeloid leukemia, and others. Acute and chronic GVHD were considered as time-dependent covariates. TBI and chronic GVHD were frequent risk factors and were always kept in the model. Risk factors for high-risk cancer sites with adequate numbers of events for analyses were also analyzed: oral cavity/pharynx, esophagus, colon, and skin. The models for high-risk cancer sites were stratified according to the primary disease as described, and patient age at transplantation (<19, 20–29, 30–39, 40–49, 50–59, and >60), and also adjusted by patient age as a continuous variable. All P-values were two-sided.

results

patient and transplant characteristics

Table 1 shows the patient characteristics, their disease, and transplant regimens for 17 545 recipients of a first HSCT. The cumulative incidences of grade 2–4 acute GVHD at 150 days and chronic GVHD at 2 years post-transplant were 35% [95% confidence interval (CI), 35%–36%] and 41% (95% CI, 40%–41%), respectively. The observation period reached 69 465 person-years among the subjects for analyses. Of the 17 545 recipients, 5864 had survived for 5 or more years, and 2192 recipients had survived 10 or more years at the time of analysis (Table 2).

incidence and types of secondary solid cancers

The cumulative incidence of solid cancers was 0.7% (95% CI, 0.6–0.9) at 5 years, 1.7% (95% CI, 1.4–1.9) at 10 years, and 2.9% (95% CI, 2.5–3.4) at 15 years after transplantation (Figure 1). Two-hundred sixty-nine solid cancers were identified. Multiple solid cancers were observed in 11 patients. Nineteen recipients were diagnosed within 1-year post-transplantation (Table 2).

risk compared with the general population

HSCT recipients had a 1.8-fold higher risk of invasive solid cancers compared with the general population (95% CI, 1.5–2.0). SIR was significantly higher for cancers of the oral cavity/pharynx (SIR = 15.7), esophagus (SIR = 8.5), colon (SIR = 1.9), skin (SIR = 7.2), and brain/nervous system (SIR = 4.1; Table 2). The risks of developing secondary cancers of the oral cavity/pharynx, esophagus, and skin were significantly higher than those in the general population throughout all periods after 1 year (Figure 2). The risk for developing colon cancer was elevated during the period of 1–4 years (SIR = 2.7), whereas the risks for developing cancer of the pancreas (SIR = 4.5) were elevated during the period of 5–9 years. Recipients were at higher risk of developing cancers of the rectum (SIR = 3.6) and the brain/nervous system (SIR = 19.1) after 10 years post-transplantation. The risk of developing secondary solid cancers of all types compared with the general population increased with the time since transplantation. This trend was observed for oral/pharynx and esophageal cancer (Table 2; Figure 2).

recipients' age at transplantation and risks for developing secondary solid cancers

SIRs were also analyzed according to the recipient's age at transplantation (Table 3). Compared with the general population in Japan, the SIRs were significantly increased for all solid cancers, oral/pharynx, esophagus, liver, bronchus/lung, and brain/nervous system for recipients who were 16–19 years of age at transplant, all solid cancers, oral/pharynx, and esophagus for recipients who

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Table 1. Patient, disease, and transplant characteristics

Characteristics	Number	Percent
Total number	17 545	
Year of transplant		
1990–1994	1630	9
1995–1999	3750	21
2000–2004	7078	40
2005–2007	5087	29
Patient sex		
Male	10 386	59
Female	7149	41
Missing	10	<1
Patient age		
Median (range)	40 (16–85)	
16–19	1399	8
20–29	3506	20
30–39	3787	22
40–49	4167	24
50–59	3549	20
≥60	1137	6
Diagnosis		
Acute myeloid leukemia	6096	35
Acute lymphoblastic leukemia	3334	19
Chronic myeloid leukemia	2514	14
Myelodysplastic syndromes	1716	10
Adult T-cell leukemia	591	3
Other leukemia	130	1
Myeloproliferative disorders	224	1
Non-Hodikin's lymphoma	1652	9
Hodikin's lymphoma	46	<1
Other lymphoma/type missing	54	<1
Multiple myeloma	210	1
Aplastic anemia	745	4
Pure red cell aplasia	4	<1
Paroxysmal nocturnal hemoglobinuria	20	<1
Solid tumor	109	1
Others	86	<1
Data missing	14	<1
Donor		
Related, siblings	7825	45
Related, other relatives	941	5
Related, data missing	179	1
Unrelated	8600	49
Stem cell source		
Bone marrow	11 866	68
Peripheral blood	3453	20
Bone marrow and peripheral blood	134	1
Cord blood	2092	12
Conditioning regimen		
Myeloablative		
Cyclophosphamide + TBI ± other	8298	47
Other TBI regimen	1321	8
Busulfan + cyclophosphamide ± other	2798	16
Other non-TBI regimen	778	4
Reduced intensity		
Fludarabine + busulfan ± other	1527	9
Fludarabine + cyclophosphamide ± other	503	3
Fludarabine + melphalan ± other	1480	8
Other RIST	631	4
Data missing	209	1
GVHD prophylaxis		
No	85	<1
Cyclosporine A + sMTX	10 091	58
Cyclosporine A ± other	1175	7
Tacrolimus + sMTX	4682	27
Tacrolimus ± other	876	5
Other	323	2
Data missing	312	2

TBI, total body irradiation; sMTX, short-term methotrexate.

were 20–29 years of age at transplant, all solid cancers, oral/pharynx, esophagus, and gallbladder for recipients who were 30–39 years of age at transplant, all solid cancers, oral/pharynx, esophagus, and skin for recipients who were 40–49 years of age at transplant, all solid cancers, oral/pharynx, esophagus, colon, and skin for recipients who were 50–59 years of age at transplant (Table 3).

risk factors for the development of secondary solid cancers

Extensive-type chronic GVHD and age at transplantation were important risk factors for the development of secondary solid cancers (Table 4). The risk was not increased in recipients who received TBI for conditioning. The results were similar when subjects were limited to those who received myeloablative conditioning (RR = 1.5, $P = 0.069$ for limited-type chronic GVHD, RR = 1.9, $P < 0.001$ for extensive-type chronic GVHD, and RR = 0.9, $P = 0.751$ for TBI). Risk factor analyses for high-risk organs with more than 10 cancer cases revealed that extensive-type chronic GVHD was an independent risk factor for cancers in the oral cavity/pharynx and esophagus. Limited-type chronic GVHD was a risk factor for cancers of skin (Table 4). For secondary cancers which developed within 1-year post-transplant, the only risk factor identified was older age at transplant (age 60 years or older; supplementary Table, available at *Annals of Oncology* online).

discussion

Our main objective was to determine the incidence of, the risk compared with the general population, and risk factors for secondary solid tumors after allogeneic stem cell transplantation in a large cohort of adult recipients. Allogeneic HCT recipients were at higher risk of developing cancers of the oral cavity, esophagus, colon, and skin. The incidence and SIR of developing all solid cancers continued to increase with follow-up, which suggested a continuous increase as follow-up progressed. Our data are important since we included a large number of subjects and person-years of follow-up, in a transplant cohort that is different from those in previously reported large studies.

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Table 2. Standard incidence ratio, ratio of observed versus expected number of secondary solid cancers according to duration post-transplant

	Time since transplantation (years)								Total		
	<1		1–4		5–9		10 or longer				
Number of recipients	17 545		10 210		5864		2192			17 545	
Person-years at risk	12 803		30 599		18 845		7218			69 465	
Secondary cancer sites	O	SIR	O	SIR	O	SIR	O	SIR	O/E	SIR	95% CI
All solid cancers	19	0.7	97	1.5*	90	2.0*	63	3.1*	269/153.6	1.8*	1.5–2.0
Oral/pharynx	0	0.0	16	9.5*	27	23.4*	21	38.5*	64/4.1	15.7*	12.1–20.1
Esophagus	0	0.0	13	6.5*	17	12.6*	11	16.8*	41/4.8	8.5*	6.1–11.5
Stomach	2	0.4	7	0.6	6	0.8	1	0.3	16/26.0	0.6	0.4–1.0
Colon	2	0.8	16	2.7*	5	1.2	4	2.2	27/14.3	1.9*	1.2–2.7
Rectum	0	0.0	1	0.2	0	0.0	5	3.6*	6/10.7	0.6	0.2–1.2
Liver	1	0.6	5	1.4	0	0.0	2	1.8	8/8.6	0.9	0.4–1.8
Gallbladder	2	5.1	2	2.1	2	3.0	0	0.0	6/2.3	2.6	1.0–5.7
Pancreas	0	0.0	2	1.0	6	4.5*	1	1.6	9/4.7	1.9	0.9–3.7
Broncus/lung	3	1.2	4	0.6	9	2.1	3	1.5	19/15.1	1.3	0.8–2.0
Skin	2	7.0	6	8.1*	3	5.7*	2	8.4*	13/1.8	7.2*	3.9–12.4
Female breast	0	0.0	3	0.3	1	0.1	3	0.9	7/24.5	0.3	0.1–0.6
Cervix uteri	1	1.3	4	2.0	1	0.7	1	1.6	7/4.8	1.5	0.6–3.0
Corpus uteri	2	3.7	1	0.7	2	1.8	0	0.0	5/3.6	1.4	0.4–3.2
Ovary	0	0.0	1	0.7	1	1.0	1	2.2	3/3.6	0.8	0.2–2.4
Prostate	1	1.2	0	0.0	1	0.6	1	1.4	3/5.4	0.6	0.1–1.6
Bladder	1	1.9	3	2.4	0	0.0	0	0.0	4/2.9	1.4	0.4–3.5
Kidney	0	0.0	1	0.6	1	0.9	0	0.0	2/4.1	0.5	0.1–1.8
Brain/nervous system	1	3.4	1	1.4	1	2.1	4	19.1*	7/1.7	4.1*	1.6–8.5
Thyroid	0	0.0	2	1.1	2	1.5	0	0.0	4/4.5	0.9	0.2–2.3
Other[a]	1		9		4		3		17		

[a]Other sites included two testicular cancers, four connective tissue cancers, four bone cancers, one larynx cancer, one malignant salivary gland tumor, one duodenum papilla cancer, one germ cell tumor, one carcinomatous pleurisy of origin unknown, and two squamous cell carcinomas of unknown origin.

*$P < 0.05$.

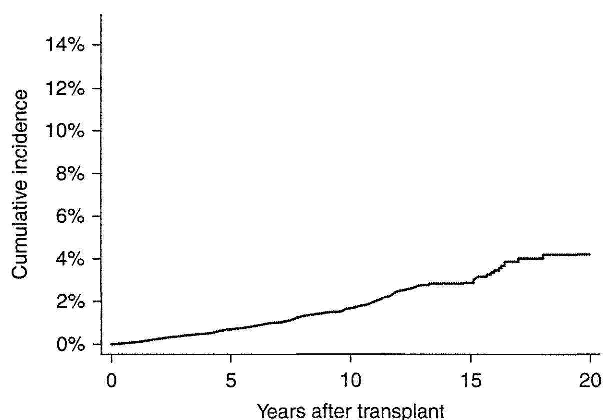

Figure 1. Cumulative incidence of developing a secondary solid cancer. The cumulative incidence of solid cancers was 0.7% [95% confidence interval (CI), 0.6–0.9] at 5 years, 1.7% (95% CI, 1.4–1.9) at 10 years, and 2.9% (95% CI, 2.5–3.4) at 15 years after transplantation.

Extensive-type chronic GVHD has repeatedly been shown to be a significant risk factor for the development of secondary solid tumor and is highly correlated with squamous cell carcinoma [8, 9, 12, 15, 16]. Extensive-type chronic GVHD was also shown to be a significant risk factor for oral cancer in our study. Extensive-type chronic GVHD was shown to be a significant risk factor for esophageal cancer, which was found to be increased in recipients compared with the general population in our study as well as in two other smaller Japanese cohorts in previous studies [11, 14]. Subjects were shown to be at a higher risk for the development of cancers of the oral cavity or esophagus at all time periods after 1 year. Data were not obtained for affected organ sites of chronic GVHD in JSHCT data collection prior to transplants in 2006. Therefore, we could not investigate whether oral or esophageal cancers were related to the chronic GVHD of the same organ. However, results of risk factor analyses for cancer sites of oral, esophagus, colon, and skin which showed high associations of extensive-type chronic GVHD and oral or esophagus cancer, limited-type chronic GVHD, and skin cancer showed that development of secondary solid tumors were likely to be influenced by GVHD-affected sites. Lifelong screening for oral, pharynx, or esophageal cancers for recipients with active or resolved chronic GVHD is important after 1-year post-transplant. The prognosis of solid cancers is highly influenced by the stage of the cancers when they are first detected. Our findings support recently published recommended screening guidelines [21, 22].

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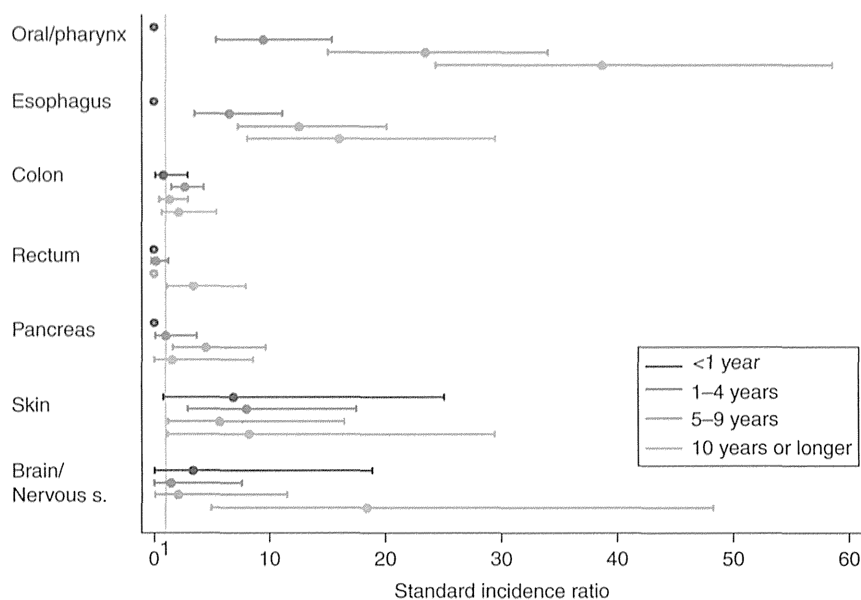

Figure 2. Trends of standard incidence ratios (SIRs) and its 95% confidence intervals (CIs) of high-risk secondary solid cancer sites according to time since transplant. The SIR and 95% CIs for <1, 1–4, 5–9, and 10 years or longer post-transplant were 0.0, 9.5 (5.4–15.4), 23.4 (15.4–34.0), and 38.5 (23.8–58.9) for oral/pharynx cancer, 0.0, 6.5 (3.5–11.2), 12.6 (7.3–20.2), and 16.8 (8.4–30.1) for esophageal cancer, 0.8 (0.1–2.9), 2.7 (1.5–4.3), 1.2 (0.4–2.9), and 2.2 (0.6–5.7) for colon cancer, 0.0, 0.2 (0.0–1.3), 0.0, and 3.6 (1.2–8.4) for rectum cancer, 0.0, 1.0 (0.1–3.7), 4.5 (1.6–9.7), and 1.6 (0.0–8.9) for pancreatic cancer, 7.0 (0.8–25.1), 8.1 (3.0–17.5), 5.7 (1.2–16.7), and 8.4 (1.0–30.3) for skin cancer, and 3.4 (0.1–19.0), 1.4 (0.0–7.7), 2.1 (0.1–11.6), and 19.1 (5.2–49.0) for cancers of brain/nervous system, respectively.

Table 3. Standard incidence ratio according to recipient's age at transplant

	Recipient's age at transplantation											
	16–19		20–29		30–39		40–49		50–59		60 or older	
Number-of-recipients	1399		3506		3787		4167		3549		1137	
Person-years at risk	7083		17 912		17 303		16 198		9126		1843	
Secondary cancer sites	O	SIR	O	SIR	O	SIR	O	SIR	O	SIR	O	SIR
All solid cancers	18	17.0*	28	4.1*	51	2.4*	71	1.4*	79	1.5*	22	1.0
Oral/pharynx	7	140.0*	11	50.7*	19	36.5*	13	10.1*	12	8.1*	2	3.9
Esophagus	1	350.0*	3	131.0*	13	48.5*	10	7.0*	13	5.9*	1	1.1
Stomach	1	13.3	0	0.0	1	0.3	7	0.8	5	0.5	2	0.5
Colon	0	0.0	0	0.0	3	2.0	6	1.3	12	2.1*	6	2.6
Rectum	1	33.1	0	0.0	0	0.0	1	0.3	4	0.9	0	0.0
Liver	1	66.4*	1	8.1	0	0.0	2	0.8	3	0.8	1	0.6
Gallbladder	0	0.0	0	0.0	2	12.0*	1	1.5	2	2.1	1	2.0
Pancreas	0	0.0	0	0.0	2	5.5	1	0.7	4	2.0	2	2.3
Broncus/lung	1	44.3*	0	0.0	2	1.6	7	1.6	7	1.1	2	0.7
Skin	1	28.6	1	6.3	0	0.0	6	11.6*	4	7.4*	1	4.0
Female breast	0	0.0	1	0.7	1	0.2	1	0.1	3	0.5	1	0.9
Cervix uteri	0	0.0	1	1.2	3	1.9	2	1.4	1	1.4	0	0.0
Corpus uteri	0	0.0	1	5.2	0	0.0	2	1.4	2	1.6	0	0.0
Ovary	0	0.0	1	3.2	0	0.0	1	0.7	0	0.0	1	6.4
Prostate	0	0.0	0	0.0	0	0.0	2	2.4	0	0.0	1	0.5
Bladder	0	0.0	0	0.0	0	0.0	2	2.3	2	1.7	0	0.0
Kidney	0	0.0	0	0.0	0	0.0	2	1.4	0	0.0	0	0.0
Brain/nervous system	2	23.9*	1	3.8	1	2.7	1	2.0	1	2.6	1	9.1
Thyroid	0	0.0	2	3.9	0	0.0	1	0.7	1	0.9	0	0.0

*$P < 0.05$.

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Table 4. Risk factors for second solid cancers among >1 year survivors after hematopoietic stem cell transplantation

Solid cancer	Risk factor	Number of patients with second cancer	RR	95% CI	*P*-value
All second solid cancers[a]		249			
	Total body irradiation	151	0.9	0.7–1.1	0.294
	Chronic GVHD				
	Limited type	45	1.4	1.0–1.9	0.087
	Extensive type	93	1.8	1.4–2.4	<0.001
	Age at transplant (years)				
	16–29	45	1.0		
	30–39	46	1.6	1.0–2.4	0.042
	40–49	68	2.5	1.7–3.7	<0.001
	50–59	71	5.5	3.7–8.2	<0.001
	60 or older	19	7.9	4.4–14.1	<0.001
Oral cancer[b]		64			
	Total body irradiation	38	1.0	0.8–1.3	0.957
	Chronic GVHD				
	Limited type	10	1.4	0.6–2.9	0.440
	Extensive type	29	2.9	1.6–5.1	<0.001
Esophageal cancer[b]		41			
	Total body irradiation	22	0.6	0.3–1.1	0.108
	Chronic GVHD				
	Limited type	7	2.1	0.8–5.9	0.151
	Extensive type	25	5.3	2.4–11.8	<0.001
Colon cancer[b]		26			
	Total body irradiation	12	0.5	0.2–1.2	0.144
	Chronic GVHD				
	Limited type	6	1.7	0.6–4.9	0.353
	Extensive type	10	1.6	0.6–4.2	0.329
	Grade 2–4 acute GVHD	12	2.0	0.9–4.4	0.101
Skin cancer[b]		13			
	Total body irradiation	12	1.2	0.8–1.6	0.377
	Chronic GVHD				
	Limited type	6	5.8	1.4–23.9	0.016
	Extensive type	2	1.8	0.3–8.9	0.500

RR, relative risk; CI, confidence interval; TBI, total body irradiation; GVHD, graft-versus-host disease.
[a]Stratified for primary disease (acute myeloid leukemia, acute lymphoblastic leukemia, chronic myeloid leukemia, and other).
[b]Stratified for primary disease (acute myeloid leukemia, acute lymphoblastic leukemia, chronic myeloid leukemia, and other) and patient age groups (<19, 20–29, 30–39, 40–49, 50–59, and >60). Adjusted for patient age as a continuous variable.

The incidence of secondary solid tumors in our study was similar to those in previously reported large studies [8, 9, 12, 13]. Rizzo et al. [12] reported that the incidence of secondary solid cancers among 28 874 transplant recipients and 85 583 person-years at risk was 1% at 10 years and 2.2% at 15 years, which were very similar to our results using the same statistical method for cumulative incidence, while treating death before secondary solid tumor as a competing risk. Majhail et al. [13] reported that the incidence of secondary solid cancers after HSCT using non-TBI, busulfan-cyclophosphamide conditioning was also ~1.2% at 10 years. The oral cavity was the most prominent high-risk cancer site compared with the general population, as in previous reports [8, 9, 12, 13]. Despite regional and racial differences in cancer incidence and cancer sites in the general population, the impact of HSCT on secondary cancer was similar.

In previous studies, TBI was reported to be a significant risk factor for the development of secondary cancer, but significant differences were not found in our study [7, 8, 10, 12, 23]. The subjects in this study were adult recipients, which may explain the different findings. Conditioning with radiation was reported to be associated with the development of secondary solid cancer in recipients at a younger age at transplant [12]. Moreover, a recent long-term follow-up analysis of patients who were transplanted after myeloablative doses of busulfan and cyclophosphamide without TBI found a similar increased incidence of secondary solid cancers as previous reports [13].

An older recipient age at transplant was a significant risk factor for the development of secondary solid tumor, as in previous studies [9, 13]. This result was not surprising since it is also the case in the general population. However, it is important to note that older patients are at higher risk of developing

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secondary cancer and to promote patient education and preventive practices, since there has been a dramatic increase in the number of transplant recipients who are more than 50 years of age at transplant over the past decade. In comparison with the general population, younger patients were at a higher risk of developing a solid tumor. Several high-risk cancer sites (esophagus, liver, and bronchus/lung) in younger group did have only one observed cases, therefore, these results should not be emphasized and need to be confirmed in other studies. These sites were found to be significant because the expected numbers in general population for these sites were extremely small.

Although this study included a large number of recipients and a large number of person-years of follow-up, there are limitations. The follow-up years for older recipients were still limited, and therefore we may find a higher incidence of and risk of secondary solid cancers among recipients who are 50 years of age or older at transplant in the future. Second limitation involves possible under-reporting by recipients to transplant centers or by transplant centers to the registry. Until recently, transplant recipients have received only limited information regarding screening or the prevention of secondary solid cancers. Another limitation of this analysis was lack of central pathology review for secondary solid tumors. JSHCT data collection does not include the submission of specimen or pathology report. Since this study included transplants from 1990, central pathology review was difficult to perform at the time of analyses. In addition, limiting secondary tumors to centrally diagnosed tumors would decrease the number of identified secondary tumors; therefore, secondary solid tumors were identified as reported from transplant centers.

In conclusion, recipients of allogeneic hematopoietic stem cell transplant had a significantly higher risk of developing secondary solid cancers than the general population. Older recipients are at higher risk of developing secondary solid tumors, as in the general population. Lifelong screening is important for high-risk organ sites, especially for oral, pharynx, and esophageal cancers in recipients with active, or a history of, chronic GVHD.

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disclosure

The authors have declared no conflicts of interest.

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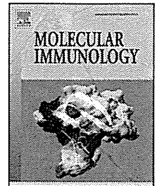

Increased serum IgA in Fcα/μR-deficient mice on the (129 x C57BL/6) F1 genetic background

Naoki Kurita, Shin-ichiro Honda*, Akira Shibuya

Department of Immunology, Institute of Basic Medical Sciences, Graduate School of Comprehensive Human Sciences, University of Tsukuba, 1-1-1 Ten-nodai, Tsukuba, Ibaraki 305-8575, Japan

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ABSTRACT

Fcα/μR (CD351) is an Fc receptor for both IgA and IgM, which is abundantly expressed in the small intestine. However, the role of Fcα/μR in the intestinal tissue is largely unknown. Here, we found that Fcα/μR is highly expressed on follicular dendritic cells (FDCs) in Peyer's patches (PP) in the small intestine. Fcα/μR-deficient mice on the (129 x C57BL/6) F1 background showed increased serum, but not fecal, IgA level in response to gut-oriented antigens. IgA+ B cells were increased in PP, but not in the lamina propria, of Fcα/μR-deficient mice, which was attenuated after reduction of commensal microbiota by oral treatment with antibiotics. Analyses of bone marrow chimeric mice, in which either FDCs or blood cells or both lack the expression of Fcα/μR, suggested that FDCs, but not blood cells, were responsible for the increased serum IgA concentration in Fcα/μR-deficient mice. Moreover, Fcα/μR-deficient mice showed enhanced germinal center formation against commensal microbiota in PP. Thus, serum IgA production against gut-oriented antigens is negatively regulated by Fcα/μR on FDCs in the F1 mice.
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1. Introduction

IgA is an antibody produced most abundantly among the classes of immunoglobulins and mainly secreted to the mucosal lumen (Kerr et al., 1990; Macpherson et al., 2012). Secreted IgA prevents penetration of pathogens into the mucosal lumen, neutralizes toxins and pathogens (Monteiro and Van De Winkel, 2003), and anchors the pathogenic bacteria to the mucus, leading to down-modulation of their pro-inflammatory epitopes (Fernandez et al., 2003). In Peyer's patches (PP), antigen-stimulated B cells form germinal centers (GC) with T cell help, leading to class switch recombination to IgA (IgA-CSR) in the presence of IgA-CSR-inducing factors, such as TGF-β and retinoic acid (Cazac and Roes, 2000). IgA+ B cells then circulate through the bloodstream and home to the intestinal lamina propria via expressions of α4β7 integrin and CCR9 (Mora and von Andrian, 2009). In addition, IgA+ B cells are also generated in the intestinal lamina propria (Fagarasan and Honjo, 2004). These in situ IgA-CSR does not require T cells, but does DC (Litinskiy et al., 2002) or epithelial cells (Xu et al., 2007), which secrete IgA-CSR-inducing factors (He et al., 2007). IgA produced in the lamina propria is transported and secreted into the mucosal lumen via binding to polymeric Ig receptor (pIgR) expressed on the mucosal epithelial cells (Kaetzel, 2005). Although the molecular mechanisms for IgA secretion into the mucosal lumen have well been studied, little is known about the maintenance of IgA in the sera.

Fc receptors are expressed on immune cells and play pivotal roles in immune responses. Several Fc receptors for IgG [FcγRI (CD64), FcγRII (CD32), FcγRIII (CD16) and FcγRIV] and IgE [FcεRI and FcεRII (CD23)] have been identified and shown to be pivotal for host immunity, allergy and autoimmune diseases (Ravetch, 1997; Takai, 2002). We have previously identified an Fc receptor for both IgA and IgM (Fcα/μR) (Shibuya et al., 2000). *Fcamr* that encodes Fcα/μR was mapped on chromosome 1, in which other Fc receptor genes are closely located each other (Shibuya et al., 2000; Shimizu et al., 2001). Although Fcα/μR is abundantly expressed in the small intestine (Sakamoto et al., 2001), its role in the mucosal tissue is largely unknown. Here, we found that Fcα/μR was preferentially expressed on FDCs in PP and negatively regulate the serum IgA level in response to gut-oriented antigens.

2. Methods

2.1. Mice

Two lines of Fcα/μR-deficient mice were generated, as described previously (Honda et al., 2009). One line was E14 ES origin and backcrossed to one generation to C57BL/6J mice

* Corresponding author. Tel.: +81 29 853 3281; fax: +81 29 853 3410.
E-mail address: shonda@md.tsukuba.ac.jp (S.-i. Honda).

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[Fcα/μR-deficient mice on (129 x C57BL/6) F1 genetic background]. Another line was BALB/c ES origin and then backcrossed to C57BL/6J mice for more than 12 generations [Fcα/μR-deficient mice on BALB/c and C57BL/6 genetic backgrounds, respectively]. BALB/c and C57BL/6J mice were purchased from Clea Japan, Inc. (Tokyo, Japan). These mice were maintained under the specific pathogen free (SPF) condition. For antibiotics treatment, mice were fed with drinking water containing ampicillin 1 g/L, neomycin 1 g/L, vancomycin 0.5 g/L and metronidazole 0.5 g/L for 4 weeks (Rakoff-Nahoum et al., 2004). All experiments were performed in accordance with the guidelines of the animal ethics committee of the University of Tsukuba Animal Research Center.

2.2. Antibodies

Fcα/μR-specific monoclonal antibody (mAb) (TX61) was established previously (Cho et al., 2006). TNP-specific mouse IgA (MOPC315) was purchased from Cappel (Solon, OH, USA). mAbs against B220 (RA3-6B2), CD21/35 (7G6), CD45 (30-F11), CD138 (281-2), IgA (C10-3), and T- and B-cell activating antigen (GL-7) were purchased from Pharmingen (San Diego, CA, USA).

2.3. Immunization

Eight- to 12-week-old Fcα/μR-deficient mice of (129 x C57BL/6) F1 background and wild-type littermate were immunized p.o. via gastric tubes with 1 mg of ovalubumin (OVA, Sigma, St. Louis, MO, USA) mixed with 10 μg of cholera toxin (List Biological Laboratories, Inc., Campbell, CA, USA) once a week for three consecutive weeks, or with 50 μg of NP-LPS (Biosearch Technologies, Inc., Novato, CA, USA) once a week for five consecutive weeks. One week after the final immunization, sera and fecal contents were obtained, as described (Shi et al., 1999).

2.4. ELISA

Serum and fecal antibody concentrations were measured by ELISA by using HRP-conjugated anti-mouse Ig isotype Abs (Southern Biotechnologies, Birmingham, AL, USA) and ABTS substrate (BioFX Laboratories, Owings Mills, MD, USA). For measurement of anti-OVA Abs, plates were coated with OVA and serially diluted sera or fecal extracts were added, and developed with HRP-conjugated anti-mouse IgA Ab. Ab titers were determined by the dilution factor which showed OD_{405} twice as high as that of the background. For measurement of anti-NP Abs, plates were coated with NP-conjugated bovine serum albumin, and were developed with HRP-conjugated anti-mouse IgA Ab. For measurement of TNP-specific mouse IgA mAb concentration, plates were coated with TNP-conjugated OVA and were developed with HRP-conjugated anti-mouse IgA Ab.

2.5. Analyses for IgA clearance

After injection of 50 μg TNP-specific mouse IgA (MOPC315) via tail vein, sera were collected at indicated time point, and measured for TNP-specific mouse IgA concentration by ELISA, as described above.

2.6. Cell preparation

Cells were harvested from the bone marrow (BM), spleen, mesenteric lymph node (MLN), Peyer's patch (PP), peritoneal exudate cell (PEC) and lamina propria (LP) 1 week after intragastric injection of 100 μg of LPS. LP cells were prepared, as previously described with minor modification (Hurst et al., 1999). Briefly, after PPs were excised, intestines were cut into small pieces and digested with dithiothreitol (DTT), followed by collagenase type VIII (Sigma, St. Louis, MO, USA). Cells were collected from the digested tissues by using centrifugation with 100%–40% Percoll.

2.7. Flow cytometry analyses

The cells were stained with cychrome-conjugated anti-B220 and PE-conjugated anti-CD138 mAbs, followed by FITC-conjugated anti-mouse IgA mAb after permeabilization with Fix & Perm kit (Invitrogen, Carlsbad, CA, USA). LP cells were stained with biotin-conjugated anti-CD45 mAb, followed by APC-conjugated streptavidin with cychrome-conjugated anti-B220 mAb. Cells were then permeabilized and stained with FITC-conjugated anti-mouse IgA mAb. For analyses of germinal center (GC) B cells, cells were stained with FITC-conjugated GL7 mAb, PE-conjugated anti-B2200 mAb and biotin-conjugated-PNA, followed by APC-conjugated streptavidin. Cells were analyzed on a FACS Calibur (BD, Franklin Lakes, NJ, USA).

2.8. Immunohistochemistry

For staining GC, frozen sections were stained with biotin-conjugated PNA, followed by Alexa594-conjugated streptavidin with FITC-conjugated anti-CD21/35 mAb. For staining Fcα/μR, frozen sections were stained with biotin-conjugated anti-Fcα/μR mAb (TX61), followed by HRP-conjugated streptavidin with TSA amplification reagent (PerkinElmer, Wellesley, MA, USA) in combination with APC-conjugated anti-CD21/35 mAb.

2.9. Bone marrow chimeric mice

Bone marrow (BM) cells (1×10^7 cells) from Fcα/μR-deficient mice or wild-type littermate mice were injected intravenously via tail vein into lethally irradiated (2×0.55 Gy) Fcα/μR-deficient or control mice. Eight weeks after BM cells transfer, the blood lymphocytes were analyzed to confirm the engraftment of donor BM cells and used for analyses.

2.10. Statistics

Statistical analyses ware performed by using unpaired Student's *t*-test. The Smirnov–Grubbs test was used to exclude outliers.

3. Results

3.1. Increased serum IgA in Fcα/μR-deficient mice on the (129 x C57BL/6) F1 genetic background

Previous reports demonstrated that *Fcamr* transcript was abundantly expressed in the small intestine (Sakamoto et al., 2001). By using immunohistochemical analyses, we observed that Fcα/μR was strongly expressed on FDCs in Peyer's patches (PP) of the small intestine (Fig. 1A). Since FDCs are involved in mucosal IgA production (Suzuki et al., 2010), IgA concentration in the serum and feces was analyzed. We found that serum IgA was significantly increased in Fcα/μR-deficient mice on the (129 x C57BL/6) F1 genetic background compared with that of littermate control mice (Fig. 1B). In contrast, IgA in feces and other immunoglobulin isotypes in serum of Fcα/μR-deficient mice were comparable to those of littermate control mice (Fig. 1C and data not shown). However, both Fcα/μR-deficient mice on the BALB/c and C57BL/6 genetic backgrounds showed comparable serum IgA levels to respective control mice

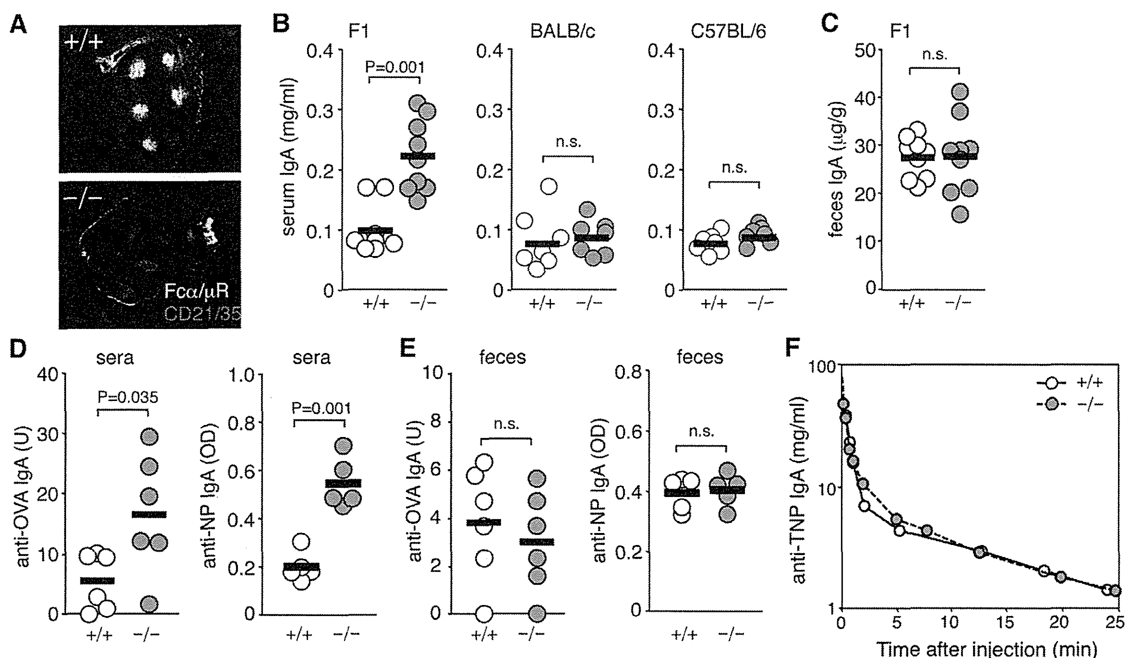

Fig. 1. Increased serum IgA in Fcα/μR-deficient mice. (A) Immunohistochemical analysis of Peyer's patches of naïve Fcα/μR-deficient on the (129 x B6) F1 genetic background (−/−) and its littermate control (+/+). (B and C) IgA concentration of serum (B) and feces (C) of naive Fcα/μR-deficient and littermate control of the F1, BALB/c, or C57BL/6 genetic backgrounds. (D and E) Antigen-specific IgA titers in the serum (D) and feces (E) in −/− and +/+ mice after oral immunization with OVA or NP-LPS, respectively. (F) The serum IgA clearance in −/− and +/+ mice. n.s., not significant.

(Fig. 1B). These results suggested that serum IgA level of Fcα/μR-deficient mice seems to be influenced by the genetic background of mice. For further analyses, we used Fcα/μR-deficient mice on the (129 x C57BL/6) F1 background and their littermates as a control. We speculated that serum IgA level was increased in response to gut-oriented antigens in Fcα/μR-deficient mice. To test this possibility, IgA production was analyzed after oral immunization of OVA and NP-LPS, T cell-dependent and independent type antigens, respectively. In response to both OVA and NP-LPS, antigen-specific IgA titer in the sera was significantly increased in Fcα/μR-deficient mice compared with that of control mice (Fig. 1D). In contrast, antigen-specific IgA titer in feces was comparable between Fcα/μR-deficient and control mice (Fig. 1E). These results indicated that serum, but not fecal, IgA titers in response to gut-oriented antigens were increased in Fcα/μR-deficient mice on the (129 x C57BL/6) F1 background. In the current study, we therefore focused on this genotype of mice to clarify how serum IgA is increased.

3.2. Normal IgA clearance in Fcα/μR-deficient mice

In mice lacking pIgR or J chain, serum IgA is increased as a result of impaired IgA transfer from the blood to the bile (Johansen et al., 1999). We speculated that increased serum IgA level in Fcα/μR-deficient mice might be a result of impaired IgA clearance. To address this question, anti-TNP mouse IgA was intravenously injected into Fcα/μR-deficient and control mice, and then measured for its concentration in the sera. Anti-TNP mouse IgA concentration was declined with time, and the half life in the sera was comparable between Fcα/μR-deficient and control mice (Fcα/μR-deficient mice, 14.7 h and control mice, 13.3 h, respectively) (Fig. 1F). Thus, the increased serum IgA in Fcα/μR-deficient mice did not result from impaired IgA clearance from the serum. Rather, the production of IgA might be increased in Fcα/μR-deficient mice.

3.3. Increased IgA$^+$ B cell populations in PP of Fcα/μR-deficient mice

To determine whether IgA production in response to gut-oriented antigens is increased in Fcα/μR-deficient mice, IgA$^+$ B cell population of lymphoid organs were analyzed by flow cytometry. We observed that IgA$^+$ B cell population (B220$^+$, IgA$^+$) was significantly larger in PPs in Fcα/μR-deficient mice than those in control mice (Fig. 2A). In contrast, IgA producing plasma cell population (B220$^-$, IgA$^+$) in lamina propria was comparable between Fcα/μR-deficient and control mice (Fig. 2A). We assumed that IgA$^+$ B cell generation might be increased in PP in response to commensal microbiota in the gut of Fcα/μR-deficient mice. To address this issue, commensal microbiota was reduced by oral treatment with antibiotics (Rakoff-Nahoum et al., 2004). As expected, serum IgA levels were down-regulated after antibiotics treatment in Fcα/μR-deficient mice (Fig. 2B). Moreover, IgA$^+$ B cell population in PPs of Fcα/μR-deficient mice was comparable to those of control mice after antibiotics treatment (Fig. 2C). These results demonstrated that the increased IgA$^+$ B cell population in PP of Fcα/μR-deficient mice was dependent on gut commensal microbiota.

3.4. Fcα/μR expressed on FDCs negatively regulate serum IgA level.

Fcα/μR was expressed most abundantly on FDCs, but also expressed on hematopoietic cells such as B cells and macrophages (Sakamoto et al., 2001). To determine the cell type responsible for the increased serum IgA level in Fcα/μR-deficient mice, we established bone marrow (BM) chimeric mice by transferring BM cells of Fcα/μR-deficient or control mice into lethally irradiated Fcα/μR-deficient or control mice. Since FDCs are radio-resistant cell population, FDCs are derived from recipient mice, whereas hematopoietic cells of BM chimeric mice are derived from

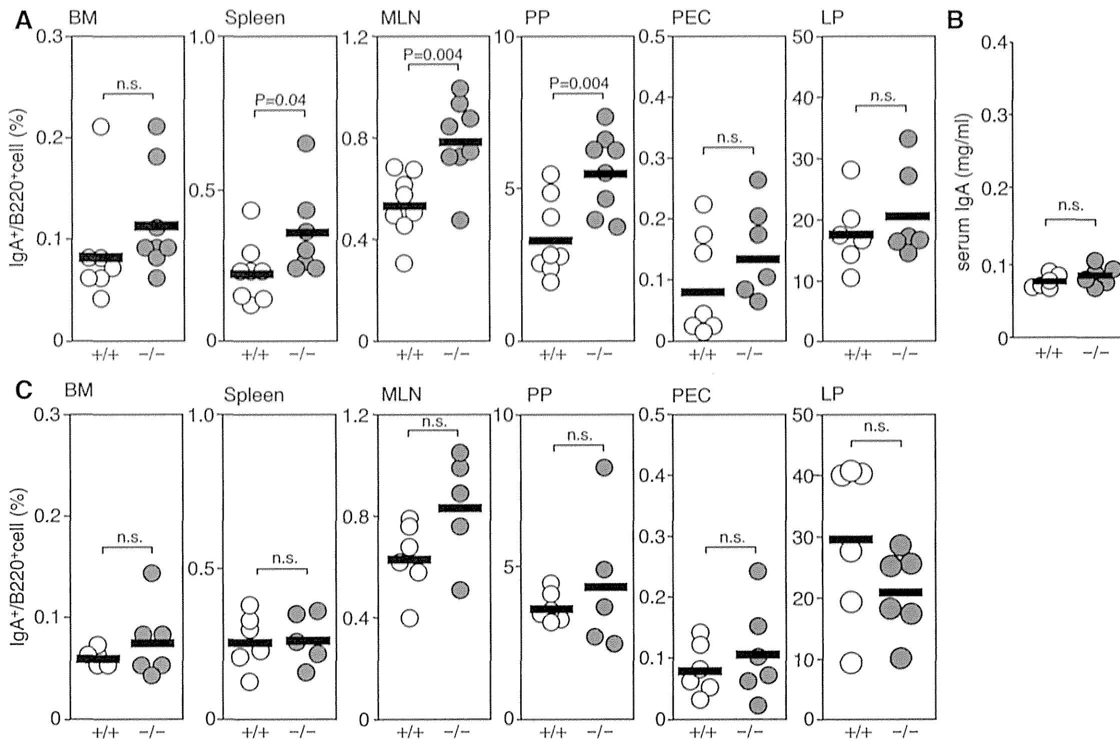

Fig. 2. Increased IgA⁺ B cell population in lymphoid organs of Fcα/μR-deficient mice. (A and C) IgA⁺ B cell populations of bone marrow (BM), spleen, mesenteric lymph node (MLN), Peyer's patches (PP), peritoneum (PEC) and lamina propria (LP) in Fcα/μR-deficient (−/−) on the F1 genetic background and littermate control mice (+/+) before (A) and after (C) oral antibiotics treatment. Data are shown as percentages of IgA⁺ B cells among total B220⁺ B cells. (B) IgA concentration in the serum of −/− and +/+ mice after oral antibiotics treatment. n.s., not significant.

transferred BM cells. We observed that only BM chimeric mice, whose FDCs, but not hematopoietic cells, lack Fcα/μR expression, showed increased IgA in the sera (Fig. 3A). Thus, Fcα/μR on FDCs is involved in the increased serum IgA in response to gut-oriented antigens.

3.5. Enhanced germinal center formation in response to commensal microbiota in Fcα/μR-deficient mice

Since FDCs contribute the generation of IgA⁺ B cell in germinal center (GC) of PP in the presence of commensal microbiota (Suzuki

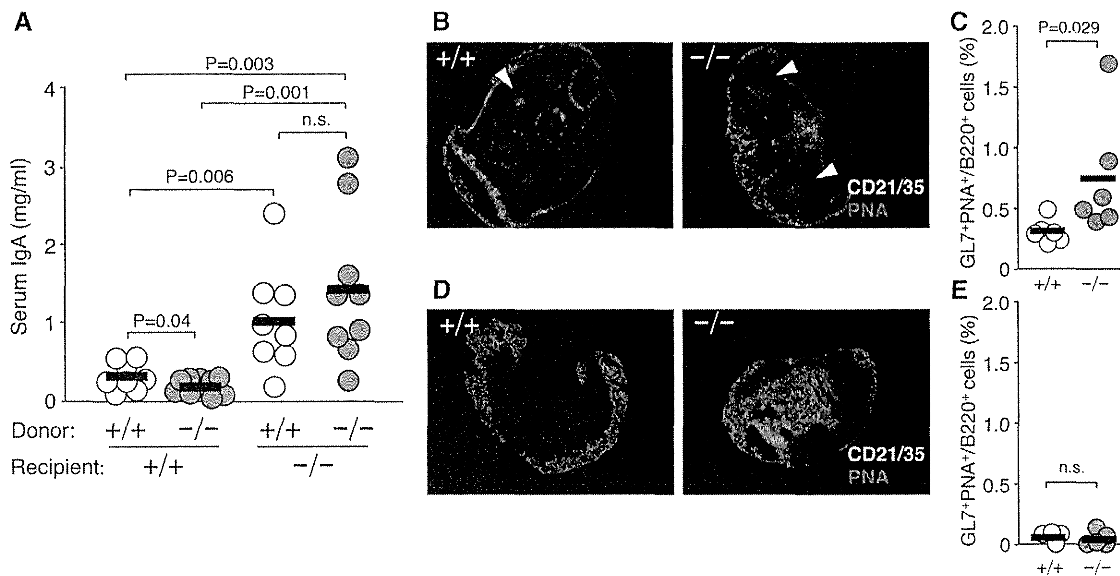

Fig. 3. Fcα/μR on FDCs negatively regulates germinal center formation of PP. (A) Serum IgA concentration of naïve bone marrow chimeric mice, which were generated by transfer of bone marrow cells from Fcα/μR-deficient mice (−/−) on the F1 genetic background or littermate control (+/+) into lethally irradiated −/− or +/+ mice. (B–E) Germinal center (GC) formation. Immunohistochemical analyses of PP of −/− or +/+ mice before (B) and after (D) oral antibiotics treatment. The white arrowhead indicates GC. The flow cytometry analysis of PP cells obtained from −/− or +/+ mice before (C) and after (E) oral antibiotics treatment. The percentage of GC B cells (GL7⁺PNA⁺B220⁺) among B cells (B220⁺) was shown. n.s., not significant.

et al., 2010), we analyzed GC formation in Fcα/μR-deficient mice. Immunohistochemical analyses showed that CD21/35⁺ PNA⁺ GC area was enlarged in PPs of Fcα/μR-deficient mice compared with control mice (Fig. 3B). These results were confirmed by flow cytometry analyses, showing that GL7⁺PNA⁺ GC B cell population was larger in PP of Fcα/μR-deficient mice than in control mice (Fig. 3C). In addition, enhanced GC formation in PP of Fcα/μR-deficient mice was diminished after reduction of commensal microbiota by antibiotics treatment (Fig. 3D and E). Thus, the increased serum IgA level in Fcα/μR-deficient mice seemed to result from increased germinal center formation in response to gut-oriented antigens.

4. Discussion

We observed that naïve Fcα/μR-deficient mice on the (129 x B6) F1 background, but not on the BALB/c or C57BL/6 genetic backgrounds, showed increased serum IgA level. It was previously reported that immune responses, including antibody production, are influenced by genetic background of mice (Dorf et al., 1974; Lipes et al., 2002). Along with this notion, involvement of Fcα/μR in serum IgA level seemed to be influenced by the genetic background of mice. Although we found that Fcα/μR on FDCs was involved in the increased serum IgA (Fig. 3A), the expression of Fcα/μR on FDCs in PP was comparable among mice on the (129 x B6) F1, BALB/c and C57BL/6 genetic backgrounds (Fig. 1A and data not shown). Thus, differential involvement of Fcα/μR in serum IgA level in each mice strain could not be explained by Fcα/μR expression on FDCs in PP. The molecular basis for this phenomenon has remained unclear.

Fcα/μR and pIgR are receptors for both IgA and IgM, which were expressed in the intestine. In addition, their genes locate very closely on chromosome 1 (Shibuya et al., 2000). However, previous work demonstrated that Fcα/μR and pIgR are differentially expressed in mucosal tissue, suggesting the different roles in this site (Wang et al., 2009). Although mice lacking pIgR showed impaired IgA clearance from the blood to the bile (Johansen et al., 1999), our analyses indicated that Fcα/μR does not contribute to IgA clearance from the blood. Rather, Fcα/μR seems to regulate serum IgA levels in response to gut-oriented antigens.

IgA⁺ B cells are generated in the presence of IgA-CSR-inducing factors in PP, and among those factors, retinoic acid induces the expression of α4β7 integrin and CCR9 on B cells surface (Mora and von Andrian, 2009). IgA⁺ B cells then circulate through the bloodstream and home to the intestinal lamina propria via α4β7 and CCR9 expression. In Fcα/μR-deficient mice, IgA⁺ B cells were increased in PP, but not in lamina propria, which is a dominant site for IgA production and secretion into feces. These results indicated that the generation of IgA⁺ B cells in PP was increased, whereas the homing of IgA⁺ B cells to lamina propria was not affected, in Fcα/μR-deficient mice. As a result, Fcα/μR-deficient mice showed increased serum IgA with normal fecal IgA in response to intestinal microbiota. Recently, it was reported that FDCs directly secrete IgA-CSR-inducing factors in response to gut microbiota for production of IgA⁺ B cells in PP (Suzuki et al., 2010). We speculate that Fcα/μR on FDCs in mice on the (129 x B6) F1 genetic background regulates production of IgA-CSR-inducing factors, except retinoic acid, in response to gut-oriented antigens in PP. In addition to cytokine secretion, FDCs capture and retain antigens for B cells activation (Heesters et al., 2014). We have shown that the retention of systemic administered T-independent antigen on splenic FDCs was prolonged in the Fcα/μR-deficient mice (Honda et al., 2009). Therefore, we also speculate that retention of gut-oriented antigens on FDCs in PP is increased in the absence of Fcα/μR, resulted in increased GC formation and IgA-CSR. The mechanisms how Fcα/μR on FDCs regulates production of IgA-CSR-inducing factors, and retention of gut-oriented antigens should be clarified in future.

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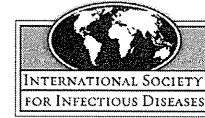

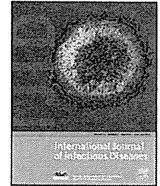

Prophylactic role of long-term ultra-low-dose acyclovir for varicella zoster virus disease after allogeneic hematopoietic stem cell transplantation

Koji Kawamura, Hidenori Wada, Ryoko Yamasaki, Yuko Ishihara, Kana Sakamoto, Masahiro Ashizawa, Miki Sato, Tomohito Machishima, Kiriko Terasako, Shun-ichi Kimura, Misato Kikuchi, Hideki Nakasone, Rie Yamazaki, Junya Kanda, Shinichi Kako, Aki Tanihara, Junji Nishida, Yoshinobu Kanda *

Division of Hematology, Saitama Medical Center, Jichi Medical University, 1-847, Amanuma-cho, Omiya-ku, Saitama-city, Saitama 330-8503, Japan

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SUMMARY

Objectives: To evaluate the prophylactic role of long-term ultra-low-dose acyclovir for varicella zoster virus (VZV) disease after allogeneic hematopoietic stem cell transplantation (HSCT).
Methods: We evaluated 141 patients who were planned to receive acyclovir at 200 mg/day until the end of immunosuppressive therapy and for at least 1 year after HSCT in our center between June 2007 and June 2012.
Results: The cumulative incidence of VZV disease after HSCT was 4.5% at 1 year and 18.3% at 2 years. Protocol violation was the only independent significant factor that increased the incidence of VZV disease (hazard ratio (HR) 7.50, 95% confidence interval (CI) 3.60–15.63). Excluding patients with protocol violation, the discontinuation of acyclovir was the only significant factor for the development of VZV disease (HR 5.90, 95% CI 1.56–22.37). Six patients experienced breakthrough VZV disease, but four of these six had not taken acyclovir for several weeks before breakthrough VZV disease. On the other hand, the cumulative incidence of VZV disease after the cessation of acyclovir was 28.4% at 1 year and 38.0% at 2 years. The proportion of disseminated VZV disease was only 7% and no patient died directly of VZV disease.
Conclusions: This study shows that long-term ultra-low-dose acyclovir appears to be effective for preventing VZV disease, especially disseminated VZV disease, after allogeneic HSCT. We recommend continuing acyclovir until the end of immunosuppressive therapy and for at least 1 year after HSCT, but additional strategies such as the administration of varicella vaccine may be needed to eradicate VZV disease.

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1. Introduction

Varicella zoster virus (VZV) disease is a common complication after hematopoietic stem cell transplantation (HSCT), with a relatively high incidence of more than 20%.[1–4] Although a localized dermatomal rash is the major clinical presentation, disseminated VZV disease occasionally occurs and may result in a fatal outcome.[3] In addition, several complications, such as post-herpetic neuralgia and secondary bacterial infections, are occasionally observed,[1,4] and may impair the patient's quality of life. Therefore, to reduce VZV disease and its complications, the long-term administration of acyclovir has been evaluated in several studies.

From the mid-1980s to the mid-2000s, several studies concluded that the overall cumulative incidence of VZV disease was not decreased by prophylactic acyclovir at 600–3200 mg/day for a fixed period of up to 6 months or 1 year, because of the increase in VZV disease after the cessation of long-term acyclovir, although VZV disease was almost completely suppressed during prophylaxis.[5–8] Therefore, the Centers for Disease Control and Prevention/Infectious Diseases Society of America/American Society for Blood and Marrow Transplantation (CDC/IDSA/ASBMT) guidelines of 2000 did not recommend universal long-term acyclovir prophylaxis to prevent VZV disease.[9] However, a large

* Corresponding author. Tel.: +81 48 647 2111; fax: +81 48 644 8167.
E-mail address: ycanda-tky@umin.ac.jp (Y. Kanda).

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retrospective study showed that acyclovir prophylaxis for 1 year reduced VZV disease, which was further decreased by the continuation of prophylaxis in patients who remained on immunosuppressive drugs.[10] Furthermore, we have previously reported that a lower dose of acyclovir at 200–400 mg/day could also reduce VZV disease.[11,12] Another clinical benefit of long-term acyclovir is the reduction of disseminated VZV disease and its complications.[10,12,13] Therefore, long-term acyclovir prophylaxis is routinely recommended for the first year after HSCT in the 2009 guidelines that were co-sponsored by several international groups.[14]

However, the optimal duration of prophylaxis, the minimal effective dose, and the risk factors for VZV disease after cessation remain unclear. In the present study, the clinical courses of patients who were planned to receive acyclovir at 200 mg/day until the end of immunosuppressive therapy and for at least 1 year after HSCT were analyzed retrospectively. First we identified risk factors predictive for the development of VZV disease. In particular, we focused on whether protocol violation was a significant risk factor or not. Next, we assessed the causal effect of acyclovir use/non-use, excluding patients with protocol violation.

2. Patients and methods

2.1. Patients

The clinical charts of 161 consecutive patients who underwent their first allogeneic HSCT between June 2007 and June 2012 at our institution were reviewed retrospectively. Among these patients, 20 were excluded: 14 died within 35 days after HSCT, five failed to achieve complete remission after HSCT, and one experienced graft rejection and early recovery of host-derived hematopoiesis after HSCT. Thus, 141 patients were included in this study (Figure 1). We followed up 102 patients until the last observation (censored observation) or the development of VZV disease, 32 patients until death without VZV disease, and seven patients until second transplantation without VZV disease. The clinical and epidemiological characteristics of the patients are shown in Table 1. This

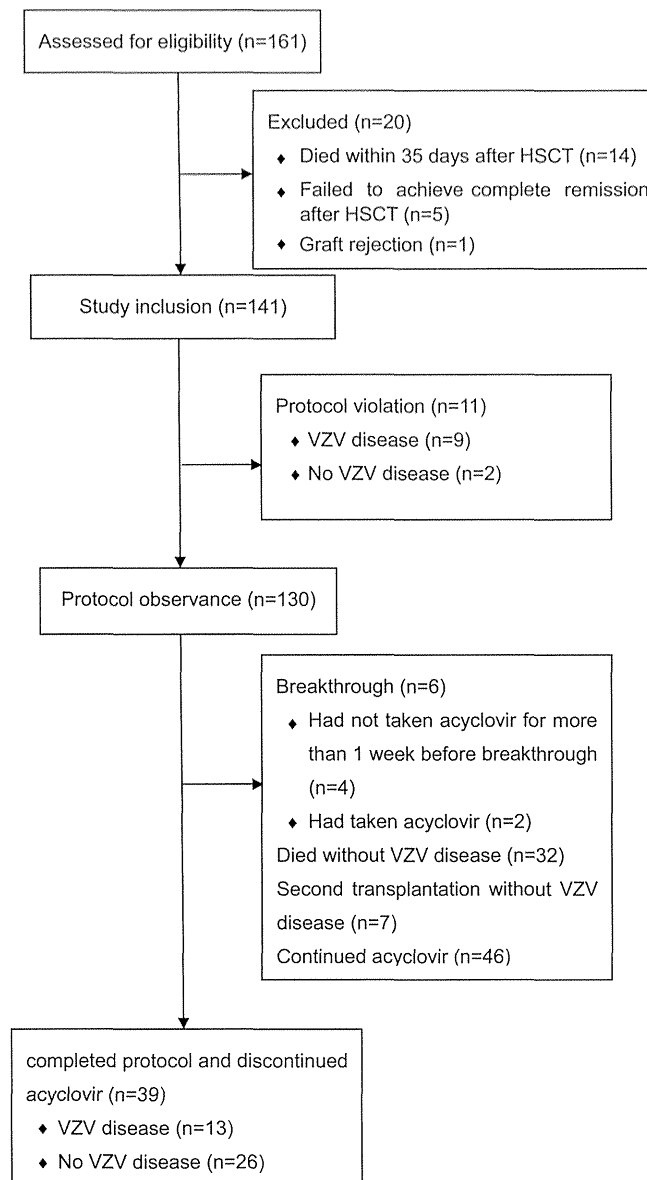

Figure 1. Flow diagram of 161 registered patients.

Table 1
Patient characteristics

Characteristics	
Median age, years (range)	45 (15–65)
Sex, *n* (%)	
Male	83 (58.9%)
Female	58 (41.1%)
Disease, *n* (%)	
AML	67 (47.5%)
ALL	14 (9.9%)
MPAL	2 (1.4%)
CML	3 (2.1%)
MDS	16 (11.4%)
NHL/ATL	23 (16.3%)
SAA	12 (8.5%)
Others	4 (2.8%)
Disease risk, *n* (%)	
Standard	111 (78.7%)
High	30 (21.3%)
Donor, *n* (%)	
Related	56 (39.7%)
Unrelated	85 (60.3%)
HLA (antigen) compatibility, *n* (%)[a]	
Matched	107 (75.9%)
Mismatched	34 (24.1%)
HLA (allele) compatibility, *n* (%)[a]	
Matched	81 (57.5%)
Mismatched	48 (34.0%)
Uncertain/missing	12 (8.5%)
Graft source, *n* (%)	
Bone marrow	73 (58.9%)
Peripheral blood	47 (33.3%)
Cord blood	11 (7.8%)
Conditioning regimen, *n* (%)	
Myeloablative	81 (57.4%)
Reduced-intensity	60 (42.6%)
GVHD prophylaxis, *n* (%)	
Cyclosporine-based	126 (89.4%)
Tacrolimus-based	15 (10.6%)
Use of ATG/alemtuzumab, *n* (%)	
Yes	19 (13.5%)
No	122 (86.5%)
Acute GVHD, *n* (%)	
Grade 0–I	90 (63.8%)
Grade II–IV	51 (36.2%)
Acute GVHD, *n* (%)	
Grade 0–II	124 (87.9%)
Grade III–IV	17 (12.1%)
Chronic GVHD, *n* (%)	
Extensive	38 (26.9%)
Limited	21 (14.9%)
None	73 (51.8%)
Not evaluable	9 (6.4%)
VZV seropositivity, *n* (%)	
Positive	135 (95.7%)
Negative	1 (0.7%)
Not examined	5 (3.6%)

AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; MPAL, mixed phenotype acute leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin's lymphoma; ATL, adult T-cell leukemia/lymphoma; SAA, severe aplastic anemia; HLA, human leukocyte antigen; GVHD, graft-versus-host disease; ATG, antithymocyte globulin; VZV, varicella zoster virus.
[a] HLA compatibility was defined according to HLA-A, HLA-B, and HLA-DR loci.

single-center retrospective analysis was approved by the Institutional Review Board of Saitama Medical Center, Jichi Medical University.

2.2. Transplantation procedure

The myeloablative conditioning regimen was mainly a combination of cyclophosphamide (60 mg/kg for 2 days) with either total body irradiation (TBI; 2 Gy twice daily for 3 days) or busulfan (3.2 mg/kg/day for 4 days). The reduced-intensity conditioning regimen was a combination of fludarabine with either busulfan or melphalan, with or without low-dose TBI, for elderly or clinically infirm patients. The conditioning regimen for severe aplastic anemia was a combination of fludarabine, cyclophosphamide, and anti-thymoglobulin, with or without TBI at 2 Gy. Alemtuzumab or anti-thymoglobulin was added for patients who received a haploidentical HSCT.[15]

Prophylaxis for graft-versus-host disease (GVHD) consisted of cyclosporine or tacrolimus combined with short-term methotrexate (10–15 mg/m^2 on day 1, 7–10 mg/m^2 on days 3 and 6, and an optional dose on day 11). The target blood concentration of calcineurin inhibitors was determined based on the disease risk. Standard-risk diseases included acute leukemia in complete remission, chronic myelogenous leukemia in the chronic phase, myelodysplastic syndrome without leukemic transformation, lymphoma in remission, and non-malignant disorders such as aplastic anemia, while more advanced diseases were considered high-risk diseases. Acute GVHD was graded as previously described.[16] Methylprednisolone or prednisolone at 1 mg/kg was added for patients who developed grade II–IV acute GVHD.

Prophylaxis against bacterial, fungal, and *Pneumocystis jirovecii* infection consisted of fluoroquinolones, fluconazole or itraconazole, and sulfamethoxazole/trimethoprim or inhalation of pentamidine, respectively. Pre-emptive therapy with ganciclovir or valganciclovir for cytomegalovirus (CMV) infection was performed by monitoring CMV antigenemia by the C10/11 method weekly after engraftment. The initial doses of ganciclovir and valganciclovir were 5 mg/kg and 900 mg once daily, respectively.[17] When increasing antigenemia was observed, the doses were elevated to 5 mg/kg and 900 mg twice daily, respectively.

2.3. Prophylactic administration of acyclovir

As prophylaxis against herpes simplex virus (HSV) infection, patients treated before August 2009 received oral acyclovir at 200 mg five times daily (ACV1000) from day −7 to 35, whereas patients treated after September 2009 received oral acyclovir at 200 mg once daily (ACV200).[18] When patients could not take acyclovir orally, intravenous acyclovir at 250 mg once and twice daily was administered instead of oral acyclovir in the ACV200 and ACV1000 groups, respectively. In both groups, oral acyclovir was principally continued at 200 mg once daily from day 36 to the end of immunosuppressive therapy and for at least 1 year after HSCT to prevent VZV disease. In the case of CMV infection or disease, acyclovir was discontinued, while ganciclovir, valganciclovir, or foscarnet was used for treatment.

2.4. Diagnosis and treatment of VZV disease

The diagnosis of VZV disease was made based on the presence of characteristic vesicular skin lesions on an erythematous base within a dermatome, or a generalized cutaneous distribution. Microbiological, pathological, and/or serological confirmation was not performed routinely except in equivocal cases. Post-herpetic neuralgia was defined as dermatomal pain persisting beyond 1 month after the initial presentation of VZV disease. VZV disease was treated with oral valacyclovir at 3000 mg/day, oral famciclovir at 1500 mg/day, oral acyclovir at 4000 mg/day, or intravenous acyclovir at 15–30 mg/kg/day for 7 days. In patients with renal impairment, the doses of these drugs were adjusted according to the creatinine clearance.

2.5. Statistical analysis

The cumulative incidence of VZV disease and the impact of possible confounding factors on VZV disease were evaluated using

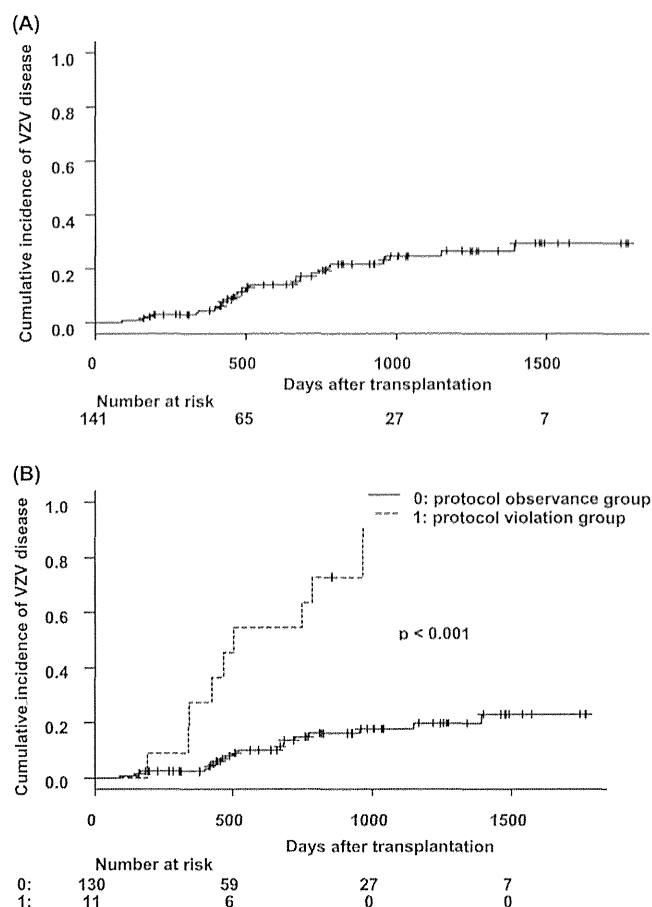

Figure 2. (A) Cumulative incidence of VZV disease after allogeneic HSCT in 141 patients who received acyclovir at 200 mg/day. (B) Cumulative incidence of VZV disease after allogeneic HSCT in 130 patients in the protocol observance group versus 11 patients in the protocol violation group.

Gray's method, while considering death without VZV disease and second transplantation as competing risks.[19] Multivariate analyses for cumulative incidences were performed using Cox proportional hazards regression modeling and Fine and Gray regression modeling.[20] In a multivariate analysis to identify risk factors predictive for VZV disease, variables subjected to the model were selected in a stepwise manner based on Akaike's information criterion (AIC). *p*-Values of less than 0.05 were considered statistically significant. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University),[21] which is a graphical user interface for R (The R Foundation for Statistical Computing, version 2.13.0). More precisely, it is a modified version of R commander (version 1.6–3) that was designed to add statistical functions that are frequently used in biostatistics.

3. Results

3.1. Administration of prophylactic acyclovir

It was planned to administer oral acyclovir until the end of immunosuppressive therapy and for at least 1 year after HSCT to prevent VZV disease. However, acyclovir was prematurely discontinued in 11 patients – at the request of the patient in two cases, at the physician's discretion in seven, and for reasons that are unclear in two. These patients were regarded as the 'protocol violation group'.

3.2. Incidence and risk factors for VZV disease after HSCT

Overall, 28 of the 141 patients developed VZV disease at a median of 486 days (range 90–1393 days) after HSCT. The cumulative incidence of VZV disease after HSCT was 4.5% (95% confidence interval (CI) 1.8–8.9%) at 1 year and 18.3% (95% CI 11.8–26.0%) at 2 years (Figure 2A). Six patients experienced breakthrough VZV disease during long-term acyclovir, at days 90, 159, 165, 398, 420, and 459 after HSCT. However, four of these six

Table 2
Risk factors for VZV disease after HSCT

Univariate analysis

Factors	Subgroup	*n*	Incidence at 3 years, % (95% CI)	*p*-Value
Age	<45 years	70	23.5 (13.1–35.7)	0.88
	≥45 years	71	25.6 (14.1–38.7)	
Sex	Male	83	20.8 (11.3–32.2)	0.56
	Female	58	29.7 (16.7–44.0)	
Disease	AML	67	20.9 (10.4–33.8)	0.96
	ALL	14	29.9 (5.1–61.2)	
	MPAL	2	NA[a]	
	CML	3	33.3 (0.1–83.2)	
	MDS	16	15.5 (2.2–40.4)	
	NHL/ATL	23	29.6 (11.2–50.8)	
	SAA	12	34.4 (6.5–65.9)	
	Others	4	25.0 (0.3–71.4)	
Disease risk	Standard	111	19.7 (11.8–28.9)	0.13
	High	30	42.6 (20.2–63.5)	
Conditioning regimen	Myeloablative	81	23.7 (13.8–35.1)	0.80
	Reduced-intensity	60	26.6 (13.7–41.4)	
Donor	Related	56	15.4 (6.6–27.6)	0.11
	Unrelated	85	32.1 (20.0–44.8)	
HLA (antigen) compatibility[b]	Matched	107	25.5 (16.3–35.6)	0.58
	Mismatched	34	21.6 (7.2–40.9)	
HLA (allele) compatibility[b]	Matched	81	24.0 (14.3–35.2)	0.92
	Mismatched	48	25.5 (9.5–45.3)	
	Uncertain/missing	12	27.8 (4.8–58.3)	
Graft source	Bone marrow	83	29.5 (18.6–41.3)	0.24
	Peripheral blood	47	13.6 (4.7–27.1)	
	Cord blood	11	NA[c]	
GVHD prophylaxis	Cyclosporine-based	126	23.4 (15.1–32.7)	0.16
	Tacrolimus-based	15	38.9 (9.4–68.5)	
Use of ATG/alemtuzumab	Yes	19	30.3 (8.6–56.0)	0.66
	No	122	23.8 (15.4–33.3)	
Protocol violation	Yes	11	NA[d]	<0.001
	No	130	17.7 (10.7–26.3)	

Multivariate analysis

Factor	Hazard ratio	95% CI	*p*-Value
Protocol violation	7.50	3.60–15.63	<0.001

VZV, varicella zoster virus; HSCT, hematopoietic stem cell transplantation; CI, confidence interval; AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; MPAL, mixed phenotype acute leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin's lymphoma; ATL, adult T-cell leukemia/lymphoma; SAA, severe aplastic anemia; NA, not available; HLA, human leukocyte antigen; GVHD, graft-versus-host disease; ATG, antithymocyte globulin.

[a] The cumulative incidence of VZV disease after transplantation was 0.0% (0.0–0.0%) at 225 days.

[b] HLA compatibility was defined according to HLA-A, HLA-B, and HLA-DR loci.

[c] The cumulative incidence of VZV disease after transplantation was 46.8% (0.4–90.2%) at 929 days.

[d] The cumulative incidence of VZV disease after transplantation was 90.9% (0.0–99.9%) at 964 days.

Table 3
Multivariate analysis treating the use of acyclovir as a time-dependent covariate, excluding patients with protocol violation

Factor	Hazard ratio	95% CI	*p*-Value
Age ≥45 years	1.18	0.33–4.25	0.80
Sex: female	1.96	0.70–5.45	0.20
Disease risk: high	2.30	0.71–7.41	0.16
Conditioning regimen: reduced-intensity	1.02	0.28–3.77	0.97
Donor: unrelated	0.89	0.16–5.16	0.90
HLA (antigen) compatibility: mismatched	0.53	0.054–5.23	0.59
Graft source: peripheral blood	0.44	0.064–3.02	0.40
Graft source: cord blood	1.40	0.090–21.54	0.81
GVHD prophylaxis: cyclosporine-based	0.69	0.14–3.44	0.65
Use of ATG/alemtuzumab	1.37	0.097–19.16	0.82
Discontinuation of acyclovir	5.90	1.56–22.37	<0.001

CI, confidence interval; HLA, human leukocyte antigen; GVHD, graft-versus-host disease; ATG, antithymocyte globulin.

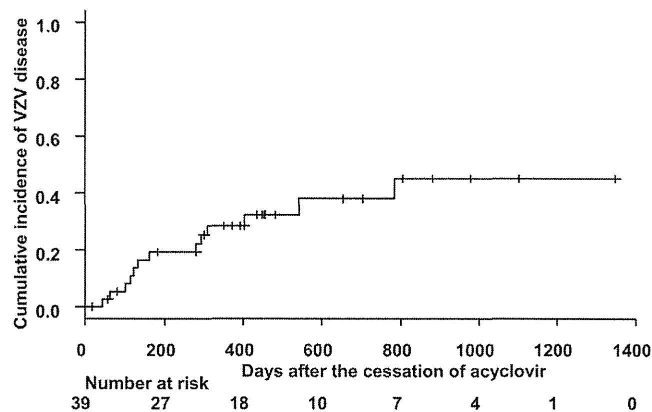

Figure 3. Cumulative incidence of VZV disease after the cessation of long-term acyclovir in 39 patients.

patients had not taken acyclovir for more than 1 week before breakthrough VZV disease due to poor compliance. The six patients developed VZV disease in a limited dermatomal distribution and responded promptly to a therapeutic dose of valacyclovir. In the multivariate analysis of the whole population, protocol violation was the only independent significant factor that increased the incidence of VZV disease (hazard ratio (HR) 7.50, 95% CI 3.60–15.63, $p < 0.001$, Table 2, Figure 2B). Nine of the 11 patients who were included in the protocol violation group developed VZV disease at a median of 464 days (range 191–964 days) after HSCT. The cumulative incidence of VZV disease at 1 year and 2 years after HSCT was 27.3% (95% CI 5.8–55.2%) and 54.5% (95% CI 20.6–79.2%), respectively, in the protocol violation group, and 2.3% (95% CI 0.6–6.1%) and 14.8% (95% CI 8.6–22.6%), respectively, in the protocol observance group ($p < 0.001$, Figure 2B). Next, we performed an analysis treating the use of acyclovir as a time-dependent covariate, excluding patients with protocol violation. The discontinuation of acyclovir was the only significant factor for the development of VZV disease (HR 5.90, 95% CI 1.56–22.37, $p < 0.001$, Table 3).

Table 4
Risk factors for VZV disease after the cessation of acyclovir

Univariate analysis				
Factors	Subgroup	*n*	Incidence at 2 years, % (95% CI)	*p*-Value
Age	<40 years	20	12.9 (1.9–34.6)	0.0066
	≥40 years	19	63.5 (26.2–85.7)	
Sex	Male	22	25.2 (8.8–45.9)	0.32
	Female	17	56.5 (16.9–83.3)	
Disease risk	Standard	32	31.3 (13.2–51.5)	0.28
	High	7	67.9 (9.8–93.7)	
Conditioning regimen	Myeloablative	27	36.3 (15.7–57.5)	0.86
	Reduced-intensity	12	37.9 (10.0–66.4)	
Donor	Related	17	21.1 (4.6–45.7)	0.14
	Unrelated	22	51.4 (21.8–74.8)	
HLA (antigen) compatibility[a]	Matched	32	37.5 (18.8–56.3)	0.96
	Mismatched	7	NA[b]	
HLA (allele) compatibility[a]	Matched	25	32.6 (17.8–55.0)	0.47
	Mismatched	9	NA[c]	
	Uncertain/missing	5	20.0 (0.4–63.2)	
Graft source	Bone marrow	23	45.0 (21.4–66.1)	0.62
	Peripheral blood	13	21.1 (2.5–51.7)	
	Cord blood	3	NA[d]	
GVHD prophylaxis	Cyclosporine-based	35	36.2 (17.8–55.0)	0.26
	Tacrolimus-based	4	NA[e]	
Duration of ACV prophylaxis	<1.5 years	28	44.9 (21.8–65.6)	0.26
	≥1.5 years	11	20.0 (2.6–49.0)	
Lymphocyte count at the cessation of ACV	$<2 \times 10^9$/l	25	34.9 (15.9–54.7)	0.71
	$\geq 2 \times 10^9$/l	14	45.6 (7.3–79.0)	
Chronic GVHD	Yes	17	48.1 (13.3–76.7)	0.71
	No	22	32.2 (12.2–54.4)	
Duration of ACV prophylaxis after the cessation of immunosuppressive drugs	<100 days	18	43.1 (15.3–68.6)	0.75
	≥100 days	21	34.0 (11.2–58.8)	

VZV, varicella zoster virus; HLA, human leukocyte antigen; NA, not available; GVHD, graft-versus-host disease; ACV, acyclovir; CI, confidence interval.
[a] HLA compatibility was defined according to HLA-A, HLA-B, and HLA-DR loci.
[b] The cumulative incidence of VZV disease after the cessation of acyclovir was 31.4% (3.0–68.3%) at 455 days.
[c] The cumulative incidence of VZV disease after the cessation of acyclovir was 35.2% (6.7–66.8%) at 455 days.
[d] The cumulative incidence of VZV disease after the cessation of acyclovir was 50.0% (0.0–96.0%) at 350 days.
[e] The cumulative incidence of VZV disease after the cessation of acyclovir was 50.0% (2.3–88.1%) at 482 days.

Table 5
Clinical outcomes of VZV disease (*n* = 28)

Localized zoster, *n* (%)	26 (93%)
Trigeminal	4
Cervical	6
Thoracic	10
Lumbar	2
Sacral	4
Disseminated disease, *n* (%)	2 (7%)
Cutaneous	2
Visceral	1[a]
Meningoencephalitis	1[b]
Hospitalized, *n* (%)	
Yes	2 (7%)
No	26 (93%)
Treatment, *n* (%)	
Acyclovir IV	1 (3.5%)
Acyclovir PO	1 (3.5%)
Valacyclovir	24 (86%)
Famciclovir	1 (3.5%)
No treatment	1 (3.5%)
Complications, *n* (%)	
Post-herpetic neuralgia	8 (29%)
Facial paralysis	1 (3.5%)
Neurologic symptoms	1 (3.5%)[c]
None	18 (64%)

VZV, varicella zoster virus; IV, intravenous; PO, by mouth.
[a] One patient had both disseminated cutaneous zoster and visceral involvement.
[b] One patient had both disseminated cutaneous zoster and meningoencephalitis.
[c] Neurologic symptoms, including paralysis in the left lower extremity, pain in both legs, and rectal/bladder disorder, persisted in one patient who developed VZV meningoencephalitis.

3.3. Incidence and risk factors for VZV disease after the cessation of acyclovir

Of the 141 patients who received long-term acyclovir, 50 discontinued acyclovir before the onset of VZV disease, death, or second transplantation. We analyzed the incidence and risk factors for VZV disease after the cessation of acyclovir in 39 of the 50 patients without protocol violation (Figure 1). The median duration of acyclovir prophylaxis was 400 days (range 364–1230 days) and the median follow-up duration after the cessation of acyclovir was 370 days (range 17–1347 days). Thirteen of the 39 patients developed VZV disease at a median of 163 days (range 44–784 days) after the discontinuation of acyclovir. The cumulative incidence of VZV disease after the cessation of acyclovir was 28.4% (95% CI 14.6–44.0%) at 1 year and 38.0% (95% CI 20.1–55.8%) at 2 years (Figure 3). In a univariate analysis, only age ≥40 years was significantly associated with a higher incidence of VZV disease (Table 4).

3.4. Clinical outcomes of VZV disease after HSCT

Twenty-six of the 28 patients developed VZV disease in a localized dermatomal distribution (Table 5). Localized zoster could be treated successfully with oral antiviral agents without hospitalization. The other two patients developed cutaneous disseminated VZV disease at days 464 and 681 after HSCT and were hospitalized for treatment. One of the two patients developed VZV meningoencephalitis at 115 days after the cessation of acyclovir and was treated with intravenous acyclovir at 30 mg/kg/day.[22] The other patient had abdominal pain with cutaneous disseminated VZV disease at 112 days after the cessation of acyclovir, and we considered it to be a symptom of visceral involvement. Although no patient died directly of VZV disease, complications after VZV disease were observed in 10 patients. Neurologic symptoms, including paralysis in the left lower extremity, pain in both legs, and a rectal/bladder disorder, persisted in one patient who developed VZV meningoencephalitis, while eight patients developed post-herpetic neuralgia and the other developed unilateral facial paralysis (Ramsay Hunt syndrome).

4. Discussion

This study demonstrated that the use of acyclovir at 200 mg/day was associated with a low incidence of VZV disease after allogeneic HSCT. On the other hand, a small number of patients experienced breakthrough VZV disease during long-term acyclovir and approximately 40% of patients developed VZV disease after the discontinuation of acyclovir. In particular, patients who had not taken acyclovir regularly due to poor compliance and who violated the protocol more frequently developed VZV disease, although we planned to administer oral acyclovir until the end of immunosuppressive therapy and for at least 1 year after HSCT. The cumulative incidence of VZV disease after HSCT was significantly higher in the protocol violation group than in the protocol observance group. These results suggest that the long-term administration of acyclovir based on our protocol was highly effective for preventing VZV disease.

However, the development of VZV disease after the cessation of long-term acyclovir remains unresolved. In a univariate analysis, only age ≥40 years was significantly associated with a higher incidence of VZV disease. However, this result might be somewhat incidental. There was only one patient who restarted immunosuppressive agents, and therefore we could not analyze the effect of the resumption of immunosuppressive therapy. Among previous studies, Erard et al. demonstrated that late-onset VZV disease was further decreased by extended prophylaxis beyond 1 year in patients who remained on immunosuppressive drugs.[10] The finding of the current study – that protocol violation was significantly associated with a higher incidence of VZV disease – is consistent with their conclusion. We cannot, however, ignore the possibility that the increased incidence of VZV disease in protocol violators was not because of the acyclovir discontinuation but because acyclovir tended to be more frequently discontinued in patients who were at higher risk for VZV disease. However, this is less likely, since the major reason for protocol violation was physician discretion, not based on the physical status of the patient. Therefore, acyclovir prophylaxis should be continued for at least as long as the immunosuppressive drugs are administered. However, the development of VZV disease after the discontinuation of acyclovir cannot be eliminated even by this strategy, and immune reconstitution against VZV may be a more important issue. Boeckh et al. demonstrated that there was no statistically significant difference in the reconstitution of VZV-specific T-cell responses between patients who received acyclovir at 800 mg twice daily and those who received placebo.[8] This finding suggests that subclinical VZV reactivation continues to occur during prophylaxis and that this antigen exposure may boost immunity and prevent subsequent symptomatic VZV disease.[23,24] However, additional strategies are required, since the natural reconstitution of VZV-specific T-cell immunity after allogeneic HSCT was not sufficient to eradicate VZV disease. A possible strategy is to administer VZV vaccine. Hata et al. demonstrated that inactivated varicella vaccine significantly reduced clinical VZV disease in patients who underwent autologous HSCT.[25] Although no data are available on the effectiveness of inactivated varicella vaccine in allogeneic HSCT recipients, several small retrospective studies have reported that the live attenuated varicella vaccine is safe and immunogenic,[26,27] and recent vaccine guidelines permit the use of a live attenuated varicella vaccine in selected patient groups.[28] Prospective studies are necessary to evaluate the safety, efficacy, and immunogenicity of the varicella vaccine.

In this study, the incidence of disseminated VZV disease was only 7% and no patient died directly of VZV disease, as found in

previous studies.[10,12,13] An important benefit of long-term acyclovir prophylaxis may be the prevention of disseminated VZV disease and its complications, although this study was too small to show such a benefit statistically. A delay in the onset of VZV disease by long-term acyclovir prophylaxis allows VZV-specific immune reconstitution, which results in a marked decrease in disseminated VZV disease.

The minimal effective dose of long-term acyclovir also remains unclear. In this study, acyclovir was administered at a dose of 200 mg, which is the minimal dose used in previous studies.[5–8,10,13] This dose was as effective at preventing VZV disease as acyclovir at higher doses in previous studies (600–3200 mg/day), and was superior in terms of low cost and drug compliance, since only one dose was required per day. On the other hand, a major concern regarding long-term ultra-low-dose acyclovir prophylaxis is the emergence of resistant VZV strains. In this study, acyclovir-resistant VZV was not observed, although there were six cases of breakthrough localized zoster that responded well to a therapeutic dose of valacyclovir. About 300 patients, including those in our previous studies, have received long-term ultra-low-dose acyclovir prophylaxis and have not developed clinically resistant VZV disease.[12] In addition, acyclovir-resistant HSV was not observed, as described previously.[18]

In conclusion, this study shows that long-term ultra-low-dose acyclovir appears to be effective for preventing VZV disease, especially disseminated VZV disease, after allogeneic HSCT. The incidence of VZV disease in patients who violated our protocol was extremely high, and therefore we recommend continuing acyclovir until the end of immunosuppressive therapy and for at least 1 year after HSCT. However, additional strategies, such as the combination of long-term acyclovir prophylaxis and the administration of varicella vaccine, may be needed to eradicate VZV disease.

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