研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
亀崎豊実	自己免疫性溶血性貧血	山口徹 北原光夫 福井次矢	今日の治療指針2008	医学書院	日本	2008	493-494
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Ⅶ.　研究成果の刊行物・別刷

（主なもの）

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ORIGINAL ARTICLE

Aberrant increase in the immature platelet fraction in patients with myelodysplastic syndrome: a marker of karyotypic abnormalities associated with poor prognosis

Naomi Sugimori[1], Yukio Kondo[1], Masami Shibayama[2], Mika Omote[2], Akiyoshi Takami[3], Chiharu Sugimori[1], Ken Ishiyama[1], Hirohito Yamazaki[1], Shinji Nakao[1]

[1]Cellular Transplantation Biology, Division of Cancer Medicine, Kanazawa University Graduate School of Medical Science; [2]Department of Clinical Laboratory, Kanazawa University Hospital; [3]Division of Transfusion Medicine, Kanazawa University Hospital, Kanazawa, Japan

Abstract

Objectives: Some patients with myelodysplastic syndrome (MDS) show a marked increase in the percentage of immature platelet fraction (IPF%) despite the absence of severe thrombocytopenia. To determine the significance of such an unbalanced increase in the IPF%, we investigated the IPF% and other laboratory findings of 51 patients recently diagnosed with MDS. *Method:* Subjects consisted of 80 healthy males, 90 healthy females, and 51 patients with MDS and 20 patients with idiopathic thrombocytopenic purpura (ITP). The IPF and IPF% were determined using a Sysmex XE-2100 system loaded with IPF Master software (XE IPF Master, Sysmex). Platelet counts were measured simultaneously. *Results:* IPF% and platelet counts of these patients ranged from 1.1% to 25.1% (median, 5.3%) and from 6 to 260×10^9/L (median, 71×10^9/L), respectively. Twelve patients showed platelet counts more than 50×10^9/L with 10% or more IPF%. All of the 12 patients had chromosome abnormalities including monosomy 7 and complex abnormalities involving 7 or 5q. In the other 39 patients who did not show the aberrant IPF% increase, chromosomal abnormalities were seen only in seven patients and none of them had chromosome 7 abnormalities. The IPF% of two patients increased to more than 10% in association with the appearance of monosomy 7. *Conclusions:* These findings suggest that a high IPF% in MDS patient may be a marker for karyotypic abnormalities with a poor prognosis, including chromosome 7 abnormalities.

Key words immature platelet fraction; IPF%; monosomy 7; myelodysplastic syndromes; Sysmex XE2100

Correspondence Shinji Nakao, MD, PhD, 13-1 Takaramachi, Kanazawa, Ishikawa, Japan, 920-8640. Tel: 81 76 265 2274; Fax: 81 76 234 4252; e-mail: snakao@med3.m.kanazawa-u.ac.jp

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Reticulated platelets are RNA-rich immature platelets and are thought to reflect the ability of bone marrow to produce platelets (1). Reticulated platelets can be measured by flow cytometry using thiazole orange (2). However, the measurement of the reticulated platelets has not been included in routine examinations because of the complexity of the procedure. Furthermore, the standard method for measurement of reticulated platelets has not been established so far. Some assays use whole blood and others use platelet-rich plasma. Monoclonal antibodies specific to platelets used to delineate reticulated platelets are varying and normal counts of reticulated platelets are reported to vary from 1% to 20% (2–16).

A recent software program using the Sysmex XE2100 fully automated complete blood count analyzer (Sysmex, Kobe, Japan) has facilitated the measurement of RNA-rich immature platelets automatically. Although this instant assay lacks specificity, the measurement of the immature platelet fraction percentage in total platelets (IPF%) may be useful in ascertaining whether thrombocytopenia is caused by reduced platelet production or increased metabolic turnover because of platelet destruction. Indeed, IPF% has been successfully utilized to differentiate ITP and aplastic anemia (AA) (17).

The measurement of IPF% has also been shown to be useful in predicting platelet recovery following

myelosuppression because of chemotherapy or preconditioning for stem cell transplantation (18–21). However, few studies have focused on the clinical significance of measuring IPF% in patients with myelodysplastic syndrome (MDS).

High IPF% is usually seen in thrombocytopenic patients whose thrombopoiesis is accelerated to compensate for low platelet counts. However, some patients with MDS exhibit IPF% above 10% despite the fact that there is no or only mild thrombocytopenia. All of the first three MDS patients we observed with such an aberrant feature had chromosome 7 abnormalities and rapidly progressed to acute myeloid leukemia. We therefore hypothesized that the unbalanced increase in IPF% may represent aberrant thrombopoiesis in MDS patients with a poor prognosis. To characterize patients with MDS showing aberrantly high IPF%, we retrospectively studied IPF% and other laboratory data in 51 patients recently diagnosed with MDS and in other patients with thrombocytopenia.

Design and methods

Subjects

Subjects consisted of 80 healthy males ranging in age from 23 to 63 yr, 90 healthy females ranging in age from 21 to 56 yr, and 51 patients with MDS and 20 patients with ITP. All subjects underwent the IPF measurement at our hospital and were subsequently followed. According to the FAB classification of MDS, 35 patients had refractory anemia (RA), one patient had RA with ringed sideroblasts (RARS), 12 patients had refractory anemia with excess blasts (RAEB) and three patients had chronic myelomonocytic leukemia. All patients and healthy individuals provided oral informed consent to the measurement of IPF.

Measurement of IPF

The IPF and IPF% were determined using a Sysmex XE-2100 system loaded with IPF Master software (XE IPF Master, Sysmex). XE-2100 is a fully automated analyzer that aspirates and analyzes ethylenediaminetetraacetic acid blood samples, and prints out test results all in about 1 min. IPF and reticulocytes were stained using reticulocyte reagents (polymethylene and oxazine dyes) and then measured by flow cytometry using a semiconductor laser. A computer algorithm discriminates the mature and immature platelets by the intensity of forward scatterd light and fluorescence. Mature platelets appear as blue dots and the immature platelets are displayed as green dots, the latter constituting the IPF parameter. IPF is calculated as the ratio of immature platelet to the total number of platelets (17, 19). When the fluorescent intensity (nucleic acid content) and the forward-scattered light intensity (Forward Scatter reflecting cell size) were plotted on the X and Y axes, respectively, the RNA-rich IPF appears in the green area on the two dimensional scattergram (Fig. 1). Platelet counts were measured simultaneously.

Cytogenetic and morphological analyses

On the day of peripheral blood collection or within 1 month before or after blood collection, karyotype analyses of bone marrow aspirates based on G banding or fluorescence *in situ* hybridization (FISH) were performed. Peripheral blood and bone marrow smears were analyzed under microscopy to ascertain the morphological characteristics of platelets and megakaryocytes.

Statistical analysis

The differences between the two groups were evaluated by Student's *t*-test. The relationship between IPF% and the platelet count was estimated using the simple linear regression and correlation analyses. Survival probabilities were estimated by the Kaplan–Meier method, and the differences in survival distributions were evaluated by log-rank test.

Results

Platelet count and IPF

Platelet counts for the control group (healthy males and females) ranged from 162 to 347×10^9/L (median:

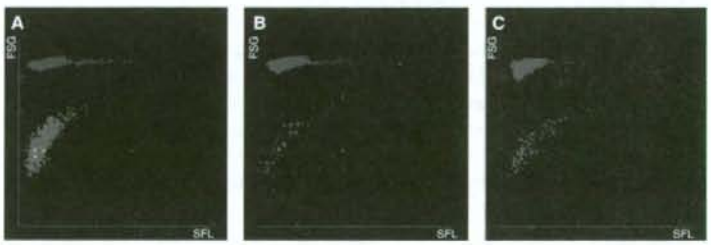

Figure 1 Measurement of immature platelet fraction (IPF). Examples of IPF scattergrams are shown. (A) a healthy individual with normal IPF%; (B) idiopathic thrombocytopenic purpura patient with IPF% of 19.8%; (C) myelodysplastic syndrome patient with IPF% of 29%. The green area indicates the IPF whereas the blue one indicates mature platelets.

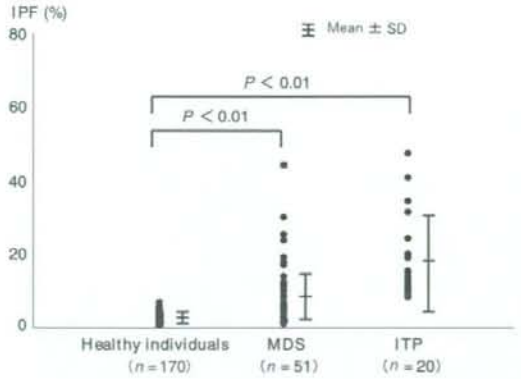

Figure 2 Immature platelet fraction (IPF) % in healthy individuals and patients with thrombocytopenia. IPF% values as determind by the blood analyzer are plotted for healthy individuals, patients with myelodysplastic syndrome and patients with idiopathic thrombocytopenic purpura.

237×10^9/L), compared with 2 to 49×10^9/L for ITP patients (median: 21×10^9/L) and from 6 to 297×10^9/L for MDS patients (median: 73×10^9/L). The mean (±SD) IPF% for the control group was 2.07 ± 1.06%, while the values for the other groups were significantly higher, at 8.80 ± 8.09% for MDS patients and 18.1 ± 11.5% for ITP patients ($P < 0.01$) (Fig. 2). IPF% was normal (0.51–4.72%) in 19 of the 51 MDS patients while 16 patients showed > 10% IPF%. Of these 16 patients, 12 patients had $\geq 50 \times 10^9$/L platelets.

Correlation between IPF% and platelet count

The correlation between IPF% and platelet count was examined for healthy individuals, ITP and MDS patients (Fig. 3). There was an inverse relationship between the two parameters in healthy individuals and ITP patients, but not in MDS patients. Some MDS patients (inside the ellipse) displayed high IPF% despite the presence of normal platelet counts. There was no apparent difference in the IPF distribution on scattergram between ITP and MDS patients with high IPF% (Fig. 1).

Clinical features of patients with platelet counts $\geq 50 \times 10^9$/L and IPF% ≥10% (Table 1)

Clinical features were compared between 12 patients with $\geq 50 \times 10^9$/L platelet count and ≥10% IPF% (high IPF% patients) and those who do not meet these criteria (control patients). Chromosomal abnormalities were found in all of the 12 high IPF% patients, but in only seven of the 39 control patients. There were no differences in age, gender, WBC, Hb, platelet count, or the proportion of advanced MDS (RAEB) between the two groups. Table 1 shows the clinical characteristics of these 12 patients. Notably five patients had monosomy 7, and another patient had complex abnormalities involving chromosome 7. Three patients showed complex abnormalities including 5q deletion. Chromosomal abnormalities in the control patients were trisomy 8 in two, 20q deletion in one, 5q deletion in one, trisomy 9 in one, 48, XY +Y,+Y in one and 47, XY, del(20)(q11),+21 in one. The patient in the control group showing only 5q deletion also showed a normal platelet count (140×10^9/L) and low IPF% (2.5%).

Morphological examination of blood and bone marrow smears showed marked variation in the platelet size in six of 12 (50%) patients and a ratio of 10% or more micro or small megakaryocytes to total megakaryocytes in 10 of 12 (83%) of the high IPF%

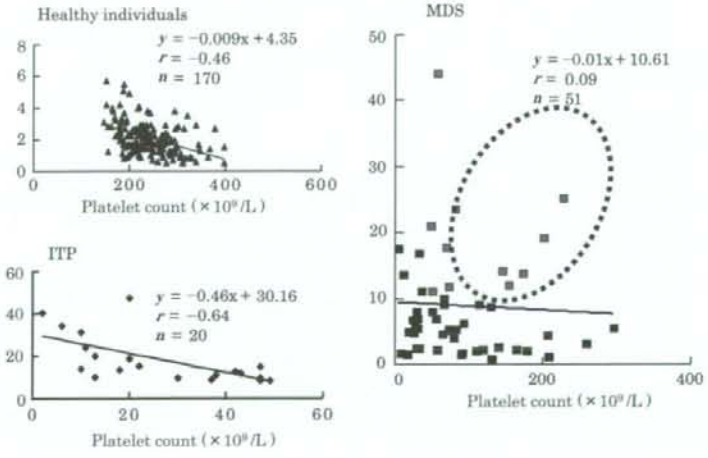

Figure 3 Correlation between immature platelet fraction (IPF) % and platelet count. IPF% and platelet count of the three different groups are plotted. X axis, platelet count; Y axis IPF%. The dotted ellipse denotes samples showing an aberrant correlation. The patients showing aberrant IPF% with higher platelet are depicted in red.

Table 1 Clinical features of patients with platelet count ≥50 × 10^9/L and IPF% ≥10%

	Case	Age/ sex	IPF%	Platelet count (×10^9/L)	Chromosomal aberration	WHO classification IPSS
1	RAEB	70 F	44.1	57	46,XX,del(1)(p),del(5)(q) 18/20 46,XX 2/20	RAEB 1 Int-2
2	RA	59 M	29.0	50	44,XY,add(1)(q32),add(5)(q11.2),add(6)(p21), −7,−8,9,add(9)(q22),10,add(11)(q23),add(12)(p11.2),add(14)(q32),−17, add(17)(q21),+mar1, +mar2, +mar3	RCMD Int-2
3	RAEB	51 F	25.1	229	46,XX, −1, add(4)(q21), −5, der(7)add(7)(p22)del(7)(q32), −11, +3mar 11/20 92,XXXX, −1, −1, add(4)(q21)×2, −5, −5, der(7)add(7)(p22)del(7)(q32) ×2, −11, −11, +6mar 1/20 46,XX 8/20	RCMD Int-2
4	RA	71 F	23.5	82	44,XX,add(5)(q11), add(6)(q13), −7, add(11)(q11), −12, del(16)(p11), −17, −17, del(20)(q11), +2mar 14/20 46,XX 6/20	RCMD Int-2
5	RARS	65 F	20.9	50	45, XX, der(3;12)(q10;10), del(5)(q13q31), −18, add(18)(p11), −20, +21, +22 5/2 47, XX, del(5)(q13q31), −18, add(18)(p11), +19, −20, +22, +mar 9/20 46, XX 6/20	RCMD-RS Int-2
6	RA	77 M	19.0	204	45, XY, −7 15/20 46, XY 5/20	RA Int-1
7	RA	80 M	14.0	147	46, XY, del(5)(q) 4/20 46, XY, del(5)(q), del(20)(q11.2q13.3) 3/20 46, XY 13/2	RCMD Int-1
8	RAEB	67 M	13.7	175	44, XY, add(5)(q11), −7, add(12)(p11), −18 1/20 44, XY, add(5)(q11), −7, add(8)(p11), add(12)(p11), −18 12/20 44, XY, add(5)(q11), −7, add(12)(p11), der(15;17)(q10;q10), −18, +mar 2/20 45, XY, add(5)(q11), −7, add(12)(p11), −18, +mar 3/20 46, XY 2/20	RAEB-1 Int-2
9	RAEB	52 M	12.1	51	45, XY, add(5)(q11), −7, add(17)(p11), −20, del(22)(q11), +mar 4/20 45, XY, add(5)(q11), −7, add(17)(p11), add(20)(q13), del(22)(q11), +mar 1/20 45, XY, add(5)(q11), −7, add(17)(p11), −18, add(20)(q13), del(22)(q11), +mar 1/20 43, XY, dic(3;14)(p11;p11),add(5)(q11), −7, add(17)(p11), −20, −22, +mar 1/20 46, XY 13/20	RCMD Int-1
10	RAEB	59 F	11.9	156	45, XX, −18, +mar 20/20	RAEB-1 Int-1
11	RA	78 F	11.8	73	45,X,-X 20/20	RCMD Int-1
12	RA	60 M	11.1	50	46,XY, add(12)(p11) 20/20	RCMD Int-1

patients. These abnormalities were found only in four of the 39 (10%) control patients. The mean platelet distribution width (PDW), an indicator of variability in platelet particle size distribution and mean platelet volume (MPV) were evaluated in 34 patients with MDS. These values were significantly higher in the high IPF% group than in the control group (PDW, 20.2 fl vs. 11.3 fl and MPV, 12.0 fl vs. 10.2 fl), suggesting the presence of prominent dysthrombopoiesis in MDS patients with high IPF%.

Change in IPF% associated with the appearance of chromosome 7

Two patients with RA showed changes in IPF% over the observation period in this study. In one patient who showed a gradual increase in the IPF%, monosomy 7 newly appeared, and the patient's RA subsequently evolved into RAEB (Fig. 4a). In another patient who responded to metenolone acetate and menatetrenone, IPF% gradually decreased from 29.0% to 9.0% in association with a decrease in the percentage of monosomy 7

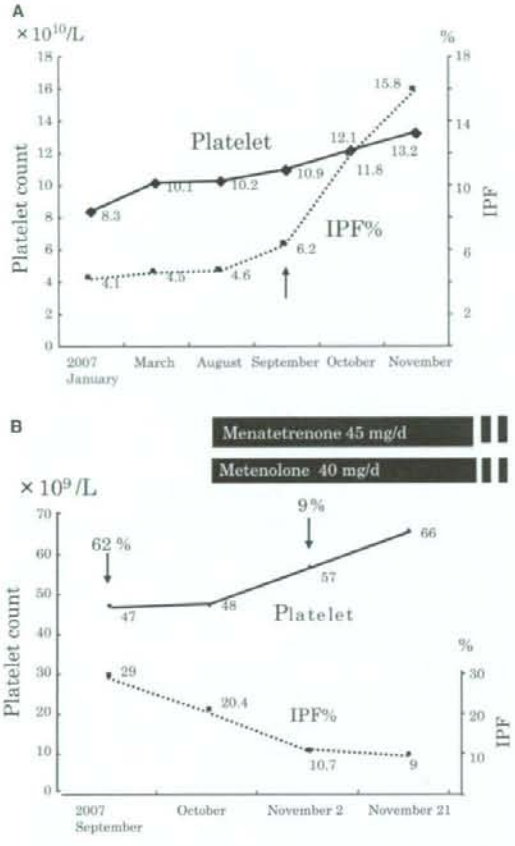

Figure 4 Changes in immature platelet fraction (IPF) % associated with the emergence or decrease of monosomy 7 clones. (A) A 22-yr-old woman with RA who originally had chromosome abnormalities, including der(1;18) showed an increase in IPF% when a monosomy 7 clone appeared. The arrow indicates the time when the monosomy 7 was first detected. (B) A 59-yr-old man with RCMD (case 2 in Table 1) with complex chromosome abnormalities including monosomy 7 who showed a decrease in IPF% in association with an improvement of pancytopenia after treatment with metenolone acetate and menatetrenone. The ratio of monosomy 7 clones as determined by FISH at the times indicated by the arrows showed a decrease from 62% to 9%.

clone from 62% to 9.2% as demonstrated by FISH analysis (Fig. 4b).

Relationship of high IPF% with prognosis

The overall survival rate was compared between the high IPF% group and the control group. As shown in Fig. 5, the patients with high IPF% show a lower survival rate than the control patients although the difference was not significant.

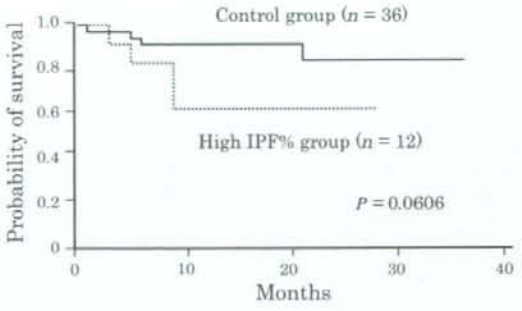

Figure 5 Kaplan–Meier analysis of the probability of overall survival (OS) in patients with myelodysplastic syndrome. OS was defined as the interval from the day of first measurement of IPF% to death or the last examination.

Discussion

IPF% has been successfully used for discrimination of pathophysiologies underlying thrombocytopenia (17, 18). Few studies, however, focused on IPF% in patients with MDS. The present study showed significantly higher IPF% in MDS patients than in healthy individuals, though there was a great range of IPF% values in MDS patients. In keeping with previous reports showing that a high IPF% reflects accelerated thrombopoiesis, there was an inverse correlation between IPF% and the platelet count in healthy individuals or that in patients with ITP. In contrast, some MDS patients showed a positive correlation between IPF% and the platelet count. Moreover, all patients with a high IPF% and a platelet count $>50 \times 10^9$/L showed chromosomal abnormalities including chromosome 7 abnormalities. The absence of monosomy 7 or chromosome 7 abnormalities in the other 39 patients suggests that an aberrant increase in IPF% represents a marker for the presence of chromosomal abnormalities with poor prognosis. The increase in IPF% in association with the emergence the monosomy 7 in one patient and a decrease in IPF% associated with a decrease in the monosomy 7 clone in another patient support this hypothesis.

Most patients with high IPF% showed marked high platelet size variation, and both MPV and PDW in these patients tended to be higher than those in MDS patients without high IPF as well as in healthy adults. The signs of dysmegakaryopoiesis such as micromegakaryocytes and isolated multinucleated megakaryocytes were commonly seen in MDS patients with high IPF%. Thus, the aberrantly high IPF% in MDS patients probably reflects the presence of dysthrombopoiesis. A recent report by Dolan *et al.* revealed prominent dysmegakaryopoiesis in patients with monosomy 7. This may account for the high incidence of monosomy 7 in patients with high IPF% (22).

According to the international prognostic scoring system (IPSS), all MDS patients with ≥20% IPF were classified as Int-2 (Table. 1), and the overall survival of high IPF% patients tended to be lower than that of control patients (Fig. 5). It is therefore possible that low-risk MDS patients considered at low risk according to IPSS may actually be at high risk for progression to leukemia if IPF% is high. IPF% can be measured very quickly using a fully automated blood cell analyzer. An increase in IPF% could be the first sign of developing 7 monosomy in patients being followed up for low-risk MDS or AA. Thus, incorporating IPF assessment into the routine examination of patients with bone marrow failure as well as in the general check-ups for healthy individuals may be useful for early identification of MDS patients with poor prognosis. Our findings warrant a prospective clinical study on MDS patients to ascertain the significance of IPF% in the management of MDS.

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Brief report

Expansion of donor-derived hematopoietic stem cells with *PIGA* mutation associated with late graft failure after allogeneic stem cell transplantation

*Kanako Mochizuki,[1] *Chiharu Sugimori,[1] Zhirong Qi,[1] Xuzhang Lu,[1] Akiyoshi Takami,[1] Ken Ishiyama,[1] Yukio Kondo,[1] Hirohito Yamazaki,[1] Hirokazu Okumura,[1] and Shinji Nakao[1]

[1]Cellular Transplantation Biology, Division of Cancer Medicine, Kanazawa University Graduate School of Medical Science, Ishikawa, Japan

A small population of CD55$^-$CD59$^-$ blood cells was detected in a patient who developed donor-type late graft failure after allogeneic stem cell transplantation (SCT) for treatment of aplastic anemia (AA). Chimerism and *PIGA* gene analyses showed the paroxysmal nocturnal hemoglobinuria (PNH)–type granulocytes to be of a donor-derived stem cell with a thymine insertion in *PIGA* exon 2. A sensitive mutation-specific polymerase chain reaction (PCR)–based analysis detected the mutation exclusively in DNA derived from the donor bone marrow (BM) cells. The patient responded to immunosuppressive therapy and achieved transfusion independence. The small population of PNH-type cells was undetectable in any of the 50 SCT recipients showing stable engraftment. The de novo development of donor cell–derived AA with a small population of PNH-type cells in this patient supports the concept that glycosyl phosphatidylinositol–anchored protein–deficient stem cells have a survival advantage in the setting of immune-mediated BM injury. (Blood. 2008;112:2160-2162)

Introduction

Although small populations of CD55$^-$CD59$^-$ blood cells are often detectable in patients with aplastic anemia (AA), it remains unclear how such paroxysmal nocturnal hemoglobinuria (PNH)-type cells arise.[1] We recently encountered a patient with immune-mediated late graft failure (LGF) following allogeneic stem cell transplantation (SCT) for treatment of AA. Analyses of the patient's peripheral blood (PB) and bone marrow (BM) showed hematopoietic stem cells (HSCs) of donor origin with mutant *PIGA*, supporting the concept that glycosyl phosphatidylinositol–anchored protein (GPI-AP)–deficient stem cells have a survival advantage in the setting of immune mediated BM injury.

Methods

Patients

A 59-year-old man underwent allogeneic PBSCT from a human leukocyte antigen (HLA)–matched sibling donor after conditioning with fludarabin (120 mg/m^2), cyclophosphamide (1200 mg/m^2), and antithymocyte globulin (60 mg/kg) for treatment of very severe AA in April 2002 (Table 1) and achieved complete donor chimerism with normal blood cell counts. In January 2006, he developed pancytopenia and was diagnosed as having LGF without residual recipient cells. The patient underwent a second PBSCT from the original donor without preconditioning on February 8, 2006. Pancytopenia resolved completely by day 16 after PBSCT. However, at approximately day 60, the blood counts decreased gradually, and the patient became transfusion-dependent. On day 196 after the second PBSCT, the white blood cell (WBC) count was 5.3×10^9/L with 17% neutrophils, the hemoglobin concentration was 75 g/L, and the platelet count was 22×10^9/L. Treatment with horse antithymocyte globulin (ATG) and cyclosporine was started on day 205 after the second PBSCT. Transfusions were terminated after 88 days of the immunosuppressive therapy. Although the patient presently receives low-dose tacrolimus for treatment of chronic graft-versus-host disease, which developed 1 year after the second PBSCT, his pancytopenia has markedly improved as shown in Table 1. PB and BM of the patient were subjected to analyses of chimerism and flow cytometry to detect CD55$^-$CD59$^-$ cells and *PIGA* gene analysis.

As controls, the PB from 51 SCT recipients (48 with hematologic malignancies and 3 with AA) who achieved a complete recovery of donor-derived hematopoiesis were subjected to flow cytometric analysis for the screening of CD55$^-$CD59$^-$ cells. Of the 51 patients, 4 and 23, respectively, had acute graft-versus-host disease (GVHD) of grade II or higher and chronic GVHD at sampling.

BM aspirates were obtained from the patient's donor and 10 healthy individuals for *PIGA* gene analysis. Informed consent was obtained from all patients and healthy individuals in accordance with the Declaration of Helsinki for blood examination, and the experimental protocol for *PIGA* gene analysis was approved by our participating institutional ethics committee (No.157).

Detection of PNH-type cells

To detect GPI-AP deficient (GPI-AP$^-$), PNH-type cells, we performed high-sensitivity 2-color flow cytometry of granulocytes and red blood cells (RBCs), as described previously.[1] The presence of 0.003% or more CD55$^-$CD59$^-$CD11b$^+$ granulocytes and 0.005% or more CD55$^-$CD59$^-$glycophorin-A$^+$ RBCs was defined as an abnormal increase based on the results in 183 healthy individuals.[2]

Cell sorting and chimerism analysis

CD3$^+$cells were isolated from the PB mononuclear cells of the patient using magnetic-activated cell sorting (MACS) CD3 Microbeads (Miltenyi Biotec, Auburn, CA). The CD55$^-$CD59$^-$CD11b$^+$ granulocytes were separated from the CD55$^+$CD59$^+$CD11b$^+$ granulocytes with a cell sorter (JSAN; Bay Bioscience, Yokohama, Japan). More than 95% of the sorted cells were

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