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Case Report

Paroxysmal nocturnal hemoglobinuria with severe pancytopenia despite of cellular marrow

Jiroh NISHIMAKI, Keisuke MIYAZAWA, Makoto YAGUCHI, Masahiko SUMI, Akihiro NAKAJIMA, Kazuma OHYASHIKI.

First Department of Internal Medicine, Tokyo Medical University, Tokyo, Japan

Abstract

A 33-year-old man was referred to our hospital because of anemia. His peripheral blood cell counts showed severe pancytopenia, and laboratory findings indicating intravascular hemolysis included elevated LDH, moderate reticulocytosis, reduced serum haptoglobin levels, and hemosiderinuria. Ham's test and the sugar water test were both positive. Flow cytometry revealed that 44.7% of erythrocytes were negative for CD55. Although there had not been any episodes of hemoglobinuria, paroxysmal nocturnal hemoglobinuria (PNH) with severe pancytopenia was diagnosed. However, his bone marrow findings showed a prominent cellular marrow with sufficient number of megakaryocytes without any dysplastic features. The myeloid/erythroid ratio of marrow nucleated cells was 1.5, suggesting that his cellular marrow did not simply reflect the compensation for hemolysis. Chromosomal analysis revealed a normal karyotype. Since diagnosis of aplastic anemia-PNH syndrome was incompatible with this case, not only hemolysis but also ineffective hematopoiesis due to impaired growth and/or maturation of PNH progenitors may have caused severe pancytopenia in this case.

Introduction

Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired clonal disease characterized by chronic intravascular hemolysis, cytopenia due to bone marrow failure and an increased tendency toward thrombosis[1]. All patients with PNH studied so far have a somatic mutation in an X-linked gene, called PIG-A (phosphatidyl inositol glycan complimentation group A), which encodes for a protein involved in the biosynthesis of the glycosyl phosphatidylinositol (GPI) molecules, serving as an anchor for many cell surface proteins including CD55 (DAF) and CD59[2,3]. This mutation has been reported to occur in hematopoietic stem cells and lead to a partial or total deficiency of the PIG-A protein[4]. As a result, a proportion of blood cells is deficient in all GPI-linked proteins. The absence of GPI-anchored proteins including DAF results in enhanced susceptibility to complement, explaining the clinical symptoms of the PNH patients such as recurrent nocturnal hemoglobinuria and episodes of venous thrombosis.

Although bone marrow hypoplasia is a major cause of death in PNH, little is known about the molecular events leading to hypoplasia[5]. In addition, about 10–30% of PNH patients initially presented with aplastic anemia accompanied by pancytopenia[5,6]. We here report a patient with PNH whose bone marrow showed a cellular marrow while also showing severe pancytopenia. However, the cellular marrow findings in this case were incompatible with those of aplastic ane-

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Address correspondence to : Jiroh Nishimaki, MD., First Department of Internal Medicine, Tokyo Medical University, 6-7-1 Nishishinjuku, Shinjuku-ku, Tokyo 160-0023, Japan
Phone : 03-3342-6111 (ext. 5894), Fax : 03-5381-6651

mia (AA)-PNH syndrome[7].

Case report

A 33-year-old man was referred to our hospital because of anemia on March ■ 1999. Physical examination on admission showed a pale face but no purpura or petechiae on the skin. Blood pressure was 132/70 mmHg and pulse rate was 80/min with regular rhythm. A systolic murmur was audible (Levine 2/VI) at the apex. No hepatosplenomegaly was observed. Peripheral blood cell counts revealed severe anemia and thrombocytopenia with moderate leukopenia and relative reticulocytosis (Table 1). In addition, elevation of the serum LDH level with isozyme 1 dominant and de-

Table 1 Laboratory data

hematology			serological and immunological		
WBC	2,300/mm³		CRP	<0.3 mg/dl	
	neutro	34%			
		(absolute 782 /mm³)	IgG	1250.0 mg/dl	
	eosin	1%	IgA	215.0 mg/dl	
	baso	0%	IgM	112.0 mg/dl	
	lymph	59%	IgD	66.4 μg/ml	
	mono	6%			
			Hp	≦10 mg/dl	
RBC	96×10⁴/mm³		vitaminB_{12}	380	pg/ml
Hb	3.9 g/dl		foric acid	7.2	ng/ml
Ht	11.0%		Fe	240	μg/dl
MCV	114.6 μmm³		TIBC	365	μg/dl
Plt	0.9×10⁴/mm³		ferritin	235.9	ng/ml
Rc	4.25%	(absolute 40,800/mm³)	ferrokinetics		
			PIDT 1/2	146 min	
coagulation			% RCU	56%	
PT	12.8 sec		NAP rate	67%	
APTT	31.0 sec		score	112	
fibrinogen	126 mg/dl		sugar water test	positive	
sFDP	67 ng/dl		HAM test	positive	
			Coombs' test	negative	
			urinary analysis		
			hemosiderinuria	negative (1st time)	
				positive (2nd time)	
blood chemistry			myelogram		
AST		20 U/L	M/E=1.5/1		
ALT		13 U/L	myeloblast	0%	
LDH		944 U/L	promyelocyte	0.4%	
		LDH isozyme 1 increased	myelocyte	6%	
ALP		83 U/L	metamyelocyte	2.8%	
γ-GTP		10 U/L	stab	9.6%	
ChE		0.63 ΔpH	seg	17.6%	
TP		6.6 g/dl	eosino	1.2%	
Alb		4.6 g/dl	baso	0%	
T-Bil		0.88 mg/dl	mono	0%	
BUN		12.1 mg/dl	lymph	34%	
UA		4.8 mg/dl	proerythroblast	0%	
CRTN		0.54 mg/dl	basochromatic erythroblast	0%	
Na		142 mEq/L	polychromatic erythroblast	23.6%	
K		4.1 mEq/L	orthochromatic erythroblast	1.2%	
Cl		105 mEq/L			
Ca		8.5 mEq/L			

[abbreviation] WBC : white blood cell, RBC : red blood cell, Plt : platelet, Ht : hematocrit, MCV : mean corpuscular volume, Rc : reticulocyte, Hb : hemoglobin, neutro : neutrophil, eosin : eosinophil, baso : basophil, mono : monocyte, lymph : lymphocyte, CRP : C-reactive protein, TP : total protein, Alb : albumin, AST : asparate aminotransferase, ALT : alanine aminotransferase, γ-GTP : gammma-glutamyl transpeptitase, LDH : lactate dehydrogenase, ALP : alkaline phosphatase, T-Bil : total bilirubin, D-bil : direct bil%irubin,I-bil : indirect bilirubin, BUN : blood urea, CRTN : creatinin, Hp : haptoglobin,ChE : cholinesterase, Ig : immunoglobulin, NAP : neutrophil alkalin phosphatase stain, PIDT : plasma iron disappearance time, % RCU : red cell utility

creased haptoglobin levels all indicated some hemolysis, but there was no increase in total bilirubin. Coombs' test was negative. There were no signs of either iron deficiency or iron overload. Urinary analysis did not reveal hemosiderinuria at the initial examination. However, hemosiderinuria became positive two weeks later. The NAP score was 112 and the positivity rate was 67%, whereas a normal control showed 204 and 94%, respectively. The sugar water test and Ham's test were both positive. These data suggested PNH.

However, it is noteworthy that, although a patient showed severe pancytopenia, bone marrow biopsy specimen from the iliac crest showed a cellular bone marrow without prominent dysplasic features (Fig. 1). A chromosomal analysis of marrow cells showed a normal karyotype. The myeloid and erythroid ratio of bone marrow mononuclear cells was 1.5. A study of ferrokinetics using ^{59}Fe showed a slight prolongation of plasma iron disappearance time and a decreased iron utilization rate of erythrocytes (PIDT 1/2 ; 146 min, % RCU ; 56%). Flow cytometry of peripheral blood revealed down regulation of cell surface expressions of CD55 in erythrocytes, lymphocytes and neutrophils, respectively (Fig. 2). Because of the small proportion of CD59-negative PNH clone in peripheral erythrocytes, it was thought that no hemoglobinuria episodes had occurred before admission. It was of interest that the proportion of CD55-negative PNH erythroblasts in the bone marrow, which were detectable as CD55-negative glycophorin-A-positive mononuclear cells by flow cytometry (data not shown), was predominant compared to that of CD55-negative peripheral erythrocytes. Treatment with fluoxymesteron (20 mg/day) for 3 months did not improve the pancytopenia. Thereafter, he has received metenolone acetate (30 mg/day). This resulted in some improvement of anemia from Hb 3.7 mg/dl to Hb 5.6 mg/dl, but there was still no effect on leukopenia or thrombocytopenia. Currently, further treatment with either immunosuppressive therapy or allogenic stem cell transplantation is under consideration.

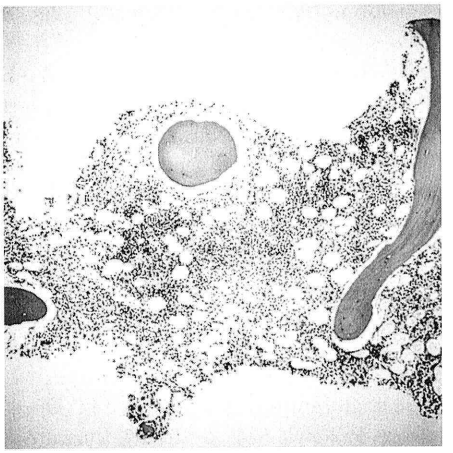

Fig. 1 A photomicrograph of the bone marrow biopsy, showing cellular bone marrow. (Hematoxylin-Eosin stain, × 100)

Discussion

It has been proposed that patients with PNH be divided into two groups : those without a history of

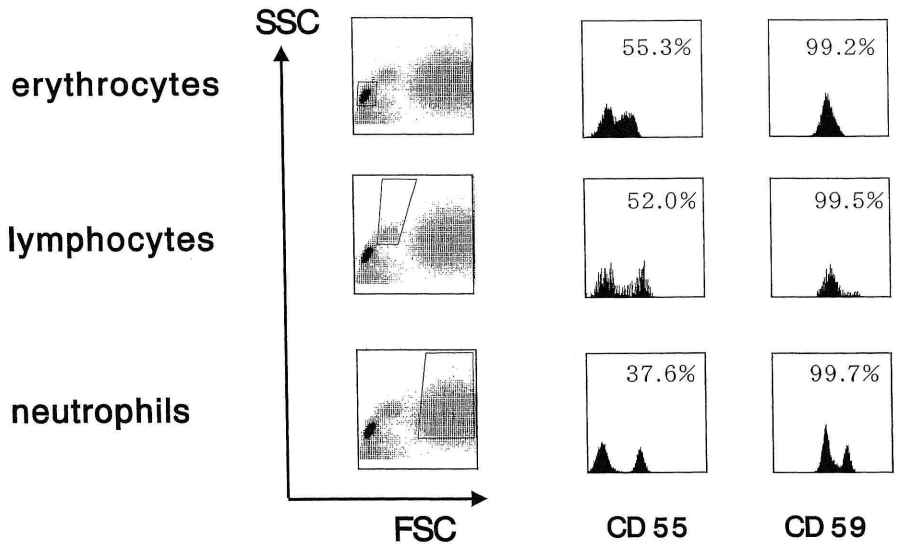

Fig. 2 Flow cytogram to detecting CD55- or CD59-positive cells in erythrocytes, lymphocytes or neutrophils. Percentages indicate relative positive cells.
SSC : side scatter, FSC : forward scatter

aplastic anemia (primary PNH) and those with a history of aplastic anemia and who subsequently developed PNH (AA-PNH syndrome)[5]. It was reported that in 220 patients with PNH, there were 65 patients (30%) in whom the diagnosis of aplastic anemia preceded that of PNH and an additional 10% of PNH patients with no history of aplastic anemia developed pancytopenia during a median follow-up of 2 years[6,7]. In the present case, although the patient initially had shown pancytopenia, a series of data such as a cellular marrow, signs of intravascular hemolysis, positivity for both Ham's test and the sugar water test, and detection of a CD55-negative clone in erythrocytes, is compatible with a diagnosis of primary PNH rather than AA-PNH syndrome[8].

Pancytopenia with cellular marrow in PNH patients was first described by Mangalik and Malaviya in 1970 9). According to their report, 46 of 116 PNH patients previously reported showed pancytopenia at some stage of their illness. Furthermore, 21 of these patients showing pancytopenia had cellular or hypercellular marrow during the phase of pancytopenia. Although, they suggested that pancytopenia associated with a cellular marrow appeared to be an important feature of PNH, the pathogenesis of this phenomenon is still not well understood.

In the present case, since the myeloid and erythroid ratio was 1.5 in the marrow, a cellular marrow was not simply because of compensation for hemolysis, a phenomenon which is usually observed in other hemolytic conditions, including autoimmune hemolytic anemia. Notably, the proportion of CD55-negative erythroblasts in marrow was more prominent than that in peripheral erythrocytes. This evidence suggests that, as often occurs in MDS[10], ineffective hematopoiesis of the PNH clone might participate in the pathogenesis of pancytopenia in primary PNH. However, it is still possible that PNH-erythrocytes may have been preferentially destroyed by complement in the peripheral blood, which may result in a lower proportion of PNH clones compared with that in bone marrow[11]. To date, there is no evidence clearly supporting the ineffective hematopoiesis of the PNH clone. Others reported that there were markedly decreased numbers of BFU-E and CFU-GM in the blood and bone marrow in patients with PNH as compared with normal control[12]. They reported that the number of "BFU-E in the PNH bone marrow is about 10% and CFU-GM is approximately one-half of normal, even in the presence of marrow hypercellularity. Others have reported that when bone marrow mononuclear cells from PNH patients were separated into CD55-positive and CD55-negative populations, only the CD55-positive population gave rise to BFU-E and CFU-E[13]. It is now well established that somatic mutations of the PIG-A gene lead to deficient expression of GPI-anchor proteins in hematopoietic cells, and this plays a causative role in the pathogenesis of PNH. However, PIG-A mutations do not explain how the defective clone can expand and become a dominant population[4,13]. Therefore, as well as in the case of MDS, several steps causing additional genetic mutations have been proposed to explain the expansion of the PNH clone in bone marrow[14]. Pancytopenia with cellular marrow as shown in this case may reflect ineffective hematopoiesis as well as some aspects of additional genetic abnormality of the PNH clone.

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正形成髄であるにもかかわらず、高度な汎血球減少であった発作性夜間血色素尿症

西巻治朗　宮澤啓介　矢口誠
住昌彦　中嶋晃弘　大屋敷一馬

東京医科大学内科学第一講座

【要旨】 症例は33歳の男性で貧血を主訴に当院に紹介された。末梢血液検査では高度な汎血球減少を呈し、LDHの上昇や中等度の網赤血球の上昇、ハプトグロビンの低値、ヘモジデリン尿などから血管内溶血を示唆した。ハムテスト、シュガーウオーターテストは陽性、フローサイトメトリーでは赤血球の13.8%がCD55陰性であった。初診に至るまで血色素尿のエピソードがなかったが、前述の結果より高度な汎血球減少を呈する発作性夜間血色素尿症（PNH）と診断した。骨髄検査の結果は、正形成で巨核球を認め、血球の異形成は認めなかった。M/Eは1.5で溶血は示唆せず、染色体分析では、正常核型であった。以上の所見よりこの症例の場合、再生不良性貧血—PNH症候群にあてはまらず、分化や増殖が障害されているPNH前駆細胞の無効造血により高度な汎血球減少を呈した可能性が高い。

〈**Key words**〉発作性夜間血色素尿症、汎血球減少、正形成髄