J Clin Immunol (2017) 37:18–21
DOI 10.1007/s10875-016-0356-4

LETTER TO EDITOR

Low T Cell Numbers Resembling T−B+ SCID in a Patient with Wiskott–Aldrich Syndrome and the Outcome of Two Hematopoietic Stem Cell Transplantations

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Received: 10 June 2016 / Accepted: 15 November 2016 / Published online: 30 November 2016

Dear Editor:

Wiskott–Aldrich syndrome (WAS) is an X-linked immunodeficiency disease associated with defective activity of WAS protein (WASP). WASP is the key regulator of actin cytoskeleton [1]; thus, any defect in WASP causes defective actin polymerization and functional abnormalities in the cells originating from bone marrow.

Clinical spectrum of loss of function mutations of the WASP gene involves the classical WAS phenotype and X-linked thrombocytopenia (XLT) [2]. Generally, WAS is associated with nonsense and frameshift mutations [2], X–linked thrombocytopenia is caused by missense mutations in exon 1 and 2 of the WASP gene leading to a decrease in WASP expression. WAS was first defined in 1937 as a novel hereditary thrombopathy. The classical phenotype was described as microthrombocytopenia, bloody diarrhea, eczema, and recurrent respiratory tract infections, especially otitis. The incidence of autoimmune manifestations and malignancies is increased in the disease. Treatment strategies of WAS range from conservative therapy to hematopoietic stem cell transplantation (HSCT) and gene therapy [3].

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Here, we report a 6-month-old boy with WAS who presented with very low numbers of T cells resembling T cell-negative, B cell-positive severe combined immunodeficiency (T−B+ SCID). He was referred to hospital with sepsis and anemia. He was the fifth child of parents who were first-degree cousins. He has been hospitalized for diarrhea and pneumonia at 1 month of age, and for sepsis and anemia at 4 months of age. On physical examination, his weight was 5,700 g (<5th percentile); height, 59 cm (<5th percentile); and head circumference, 41 cm (<5th percentile). He was pale, had tachypnea and monilial patches in his mouth, erythematous maculopapular eruption, and petechiae on his face and chest. The liver was palpable 2 cm below the costal margin. Complete blood count revealed anemia, lymphopenia, and thrombocytopenia (Table 1), and 38% normoblasts. Direct Coombs test was 2+, and the reticulocyte count, 4.82%. Bone marrow aspiration microscopy showed increased erythropoietic activity, compatible with hemolytic anemia. Lymphopenia was present and lymphocyte subset analysis was compatible with T cell-negative, B cell-positive, natural killer cell-positive severe combined immunodeficiency (T−B+NK+ SCID; Table 1). Chest X-ray and high-resolution CT showed a ground-glass appearance in both lungs. HIV serology was negative. Intravenous immunoglobulin (IVIG) and steroid treatment (2 mg/kg) were given for hemolytic anemia. Cytomegalovirus (CMV) polymer chain reaction was positive (1,325,000 copy/ml), and ganciclovir was begun. A week after ganciclovir therapy was started, bone marrow cells (4×10^8 nucleated cells/kg) were given from the patient's CMV-seropositive, HLA-identical healthy sister without any conditioning regimen at the age of 6 months (Table 2). CD3+ cells increased relatively about 15 days after HSCT. This was attributed to lymphoid engraftment. Hemolytic anemia resolved, the frequency of infections decreased, and the patient began to gain weight. A month after HSCT, IVIG treatment

Table 1 Laboratory findings before and after transplantation

	Before first HSCT	Before first HSCT	After first HSCT	After first HSCT	After second HSCT
Patient/age	4 months old	6 months old	6.5 months old	10 months old	9 years old
Complete blood count					
Hb (g/dl)	6.1	8.6	7	8.4	11.6
MCV	ND	74.9	70.3	63.9	77.7
WBC (/mm^3)	3,300	9,100	5,100	9,600	7,100
Plt (/mm^3)	28,000	32,000	24,000	24,000	269,000
ALC (/mm^3)	1,100 (3,900–9,000)	2,730 (3,900–9,000)	2,700 (3,400–9,000)	6,900 (3,400–9,000)	2,300 (1,900–3,700)
ANC (/mm^3)	495	5,460	1,100	2,100	3,800
MPV	ND	6.2	5.6	ND	6
Immunoglobulins					
Ig A (g/dl)	63.9 (13.5–72)	64 (7–123)	ND	12.8 (17–69)	<6.6 (62–390)
Ig G (g/dl)	1,310 (294–1,165)	1,310 (304–1,231)	ND	1,416 (463–1,006)	1,380 (842–1,943)
Ig M (g/dl)	118 (33–154)	118 (32–203)	ND	99.5 (46–159)	64.7 (54–392)
IgE (IU/ml)	ND	ND	ND	2.34	2.77
IVIG therapy	+	+	+	–	–
Lymphocyte subsets					
CD3 (%/mm^3)	ND	15 (50–77)	36 (50–77)	60 (54–76)	67 (55–78)
		409 (2,400–6,900)	(2,400–6,900)	4,140 (1,600–6,700)	1,541 (700–4,200)
CD4 (%/mm^3)	ND	1.3 (33–58)	17 (33–58)	8.5 (31–54)	28 (27–53)
		35 (1,400–5,100)	451 (1,400–5,100)	587 (1,000–4,600)	644 (300–2,000)
CD8 (%/mm^3)	ND	15.3 (13–26)	20 (13–26)	51 (12–28)	33 (19–34)
		417 (600–2,200)	540 (600–2,200)	3,519 (400–2,100)	759 (300–1,800)
CD19 (%/mm^3)	ND	73 (13–35)	55 (13–35)	35 (15–39)	13 (10–31)
		1,992 (700–2,500)	1,485 (700–2,500)	2,415 (600–2,700)	299 (200–1,600)
CD16+56 (%/mm^3)	ND	8.8 (2–13)	9 (2–13)	1.2 (3–17)	17 (4–26)
		240 (100–1,000)	243 (100–1,000)	83 (200–1,200)	391 (90–900)
Lymphocyte proliferation (cpm × 10^{-3}; patient/control)	ND	5.5/138	53.3/43.3	ND	25/76
PHA		3.6/131	–		25/62
ConA		54/88	–		5.6/1
PMA + ion		1.8/1.9	0.8/0.9		10/1.5

Age at first HSCT, 6 months old; age at second HSCT, 7 years old

Numbers in parenthesis are the reference values

ND not determined, *Hb* hemoglobin, *MCV* mean corpuscular value, *WBC* white blood cells, *Plt* platelets, *ALC* absolute lymphocyte count, *ANC* absolute neutrophil count, *MPV* mean platelet volume, *Ig* immunoglobulin, *IVIG* intravenous immunoglobulin, *PHA* phytohemagglutinin, *ConA* concanavalin A, *PMA* phorbol myristate acetate

was stopped. Common genetic defects causing T−B+ SCID, such as IL2RG, IL7RA, and JAK3 gene defects, were excluded by Sanger sequence analysis. There was continuing thrombocytopenia after HSCT. Low MPV, which was not recognized before HSCT, drew attention. It was thought to be due to WAS, and Sanger sequence analysis was performed, this time for the WASP gene. Analysis revealed a splice site mutation, which is predicted to result in aberrant splicing of a part of WASP transcripts (exon 11(-1) G>T). The chimerism analysis showed mixed chimerism with a 32% donor profile at 6 years of age, 5 years after the first HSCT. During the follow-up of 5 years, he continued to have severe thrombocytopenia and multiple episodes of epistaxis. Thus, second HSCT with a conditioning regimen at the age of 6 years was performed (Table 2). He received acyclovir, fluconazole, and trimethoprim–sulfamethoxazole for viral, fungal, and *Pneumocystis jirovecii* infection prophylaxis respectively. Weekly IVIG was administered at a dose of 400 mg/kg from day −1 to discharge. Intravenous glutamine, enoxiparin, ursodeoxycholic acid, and vitamin E were given for veno-occlusive disease (VOD) prophylaxis. Peripheral blood was used as a stem cell source and the CD34+ cell dose was 3.7×10^6/kg (Table 2). Neutrophil and thrombocyte engraftment were achieved on day +13 and +18 respectively. The patient did not develop acute and chronic graft

Table 2 Characteristics and results of hematopoietic stem cell transplantations

	First HSCT	Second HSCT
Age	6 months	6 years
Donor/patient gender	Male/female	Male/female
Donor	HLA identical sibling	Same as the first HSCT
Conditioning regimen	–	Busulfan (12.8 mg/kg, iv)
		Cyclophosphamide (200 mg/kg)
GVHD prophylaxis	–	Cyclosporine A + methotrexate
Stem cells source	BM	PB
CD34+ cell dose	3×10^6/kg	3.7×10^6/kg
Neutrophil engraftment day	NA	Day +13
Thrombocyte engraftment day	NA	Day +18
Chimerism analysis	32% donor profile	98.5% donor profile
	(At age 6 years, before second HSCT)	(+1 month)

GVHD graft versus host disease, *HSCT* hematopoietic stem cell transplantation, *HLA* human leukocyte antigen, *BM* bone marrow, *PB* peripheral blood, *NA* not applicable

versus host disease (GVHD) or VOD. Chimerism analysis showed full donor chimerism at +1 month after transplantation and full donor chimerism sustained during follow-up. Now, 5 years after the second HSCT, the patient is doing well with a normal count and size of platelets.

Wiskott–Aldrich syndrome protein (WASP) is a hematopoietic-specific member of a family of cytoskeletal regulators, and is found in the cytoplasm of all leukocytes [1]. Dynamic cytoskeletal changes that are essential for the maintenance of cellular homeostasis, including adhesion, migration, phagocytosis, receptor-mediated cellular activation processes, and immune synapse formation, are regulated by WASP. Defects in WASP lead to immune deficiency involving both innate and adaptive immunity [3]. Immunological findings in WAS are variable, including quantitative and qualitative T cell defects. Lymphocyte numbers tend to be normal at birth, but decline to below 1,000 cells/mm^3 in about a quarter of patients with age [4]. Decreased mitogen-induced proliferation [5], effects on regulatory T cells (Tregs) and cytokine secretion were reported [6, 7]. Rawlings et al. suggest that the accelerated destruction of lymphocytes by spontaneous apoptosis could account for the progressive deterioration of immune function in WAS patients [8]. Our patient had lymphopenia at the age of 4 months and presented with a very severe T cell deficiency, which, as far as we know, has not been reported before. Lymphocyte proliferation testing with phytohemagglutinin was also very low, and this was possibly due to immunosuppression caused by CMV and/or steroids.

The functions of B cells are also affected in WAS. Serum immunoglobulin levels are extremely variable [4]. Generally, serum IgG levels are within the normal range, whereas IgA and IgE are elevated. Decreased/normal IgM levels were reported [9]. On admission, our patient had normal IgA levels and elevated IgG. Normal IgA/IgM values do not exclude SCID [10, 11]. Therefore, we thought the diagnosis of SCID, despite normal IgA and IgM levels. The absence of low IgG was attributed to the maternal immunoglobulin transferred.

In previous surveys, approximately 30–70% of patients with WAS were reported to develop autoimmune or inflammatory manifestations [4, 12]. There are many causes contributing to autoimmunity in WAS. Decreased effector function and survival of regulatory T cells may lead to a loss of peripheral tolerance and autoimmunity. Other reasons are inefficient cytotoxic granule secretion, defective T cell apoptosis, and inefficient clearance of apoptotic bodies/immune complexes by macrophages [6]. Common autoimmune or inflammatory complications associating WAS are vasculitis, uveitis, inflammatory bowel disease, and cytopenia, most commonly autoimmune hemolytic anemia (AIHA) [12].

In WAS patients, HSCT is an appropriate treatment and is essential, especially in those with AIHA [12]. In a retrospective study, the survival prospects were better if HSCT was performed at an earlier age in patients with WAS and associated AIHA [12]. In the cohort of patients with WAS and AIHA, it was suggested that severe AIHA might have been identified before the age of 5 years and nearly all patients required HSCT.

There are important issues to be discussed about the clinical follow-up of this patient. First, the clinical presentation and laboratory findings resembled SCID. No WAS patients with such low T cell levels had been reported before. Low T cell levels were possibly due to severe T cell suppression associated with severe CMV infection.

Second, the presence of pancytopenia (anemia, low WBC, and thrombocytopenia), not only thrombocytopenia, made the clinicians think about bone marrow suppression or hemophagocytosis, which may have been triggered by CMV. Autoimmune cytopenia may be associated with all kinds of primary immunodeficiencies.

Third, the results of the first HSCT was interesting. The increase in lymphocyte subsets after the first HSCT was attributed to engraftment during follow-up, but it may be partly due to the recovery from CMV infection and its immunosuppression. In addition to antiviral therapy for CMV, healthy mature T cells given from the CMV-seropositive donor may have contributed to the resolution of CMV. In SCID patients, HSCT may be performed without a conditioning regimen as T cells are congenitally depleted. In the present patient, the first HSCT was performed without a conditioning regimen as the patient was diagnosed as T−B+ SCID. As far we know, there are no reports of a WAS case undergoing transplantation without a conditioning regimen. Interestingly, mixed donor chimerism was recorded in the patient at 6 years of age, about 5 years after the first HSCT. In SCID, the increase in donor chimerism less than 3 months after HSCT may be due to expansion of the mature T cells present in the HSC inoculum rather than thymopoiesis [13]. Thymopoiesis becomes important 3 months after the HSCT. We did not test the thymopoiesis, but the increase in chimerism in this patient with WAS may have been due to the severity of cellular immunodeficiency.

Fourth, continuing thrombocytopenia after HSCT was remarkable, and may also be seen as a form of autoimmune cytopenia in the course of acute and chronic GVHD. The definitive diagnosis of WAS in the present patient was clear only after molecular study.

Owing to severe bleeding episodes, second HSCT with a full conditioning regimen was performed 6 years after the first transplantation. Successful engraftment, including the normal number and size of platelets, was observed, and full chimerism achieved.

Another comment may be made on low MPV, which was not noticed before the first HSCT. However, the patient presented with severe CMV infection and hemolytic anemia after blood transfusions.

It is of utmost importance to remember that defects in the same gene may result in various clinical presentations and defects in different genes may result in the same clinical presentation, which complicates diagnosis, but on the other hand enlarges the clinical spectrum of diseases.

Compliance with Ethical Standards

Conflicts of Interest The authors declare that they have no conflicts of interest.

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