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ASTUTE CLINICIAN REPORT

Allogeneic Bone Marrow Transplantation Appears to Ameliorate IgA Nephropathy in a Patient with X-linked Thrombocytopenia

Akihiro Hoshino · Masaki Shimizu · Hiroyoshi Matsukura · Hisano Sakaki-Nakatsubo · Keiko Nomura · Toshio Miyawaki · Hirokazu Kanegane

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Abstract Wiskott-Aldrich syndrome (WAS) is caused by a mutation in the *WAS* gene, and it is clinically characterized by the triad of thrombocytopenia, eczema and immunodeficiency. X-linked thrombocytopenia (XLT), which is a clinically mild form of WAS, is also caused by a *WAS* gene mutation. Patients with WAS/XLT sometimes also have autoimmune diseases such as IgA nephropathy. Progression of IgA nephropathy may lead to chronic renal failure with a poor prognosis. Here, we describe an XLT patient who also had IgA nephropathy. The patient underwent bone marrow transplantation (BMT) because of an associated-lymphoproliferative disorder, and clinical and histological improvement in his IgA nephropathy was observed after BMT. The amount of galactose-deficient IgA in the patient's serum markedly decreased after BMT. Therefore, immunological reconstitution might improve autoimmune diseases in patients with WAS/XLT.

Keywords Aberrant IgA · bone marrow transplantation · IgA nephropathy · Wiskott-Aldrich syndrome · X-linked thrombocytopenia

A. Hoshino · H. Sakaki-Nakatsubo · K. Nomura · T. Miyawaki · H. Kanegane (✉)
Department of Pediatrics, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan
e-mail: kanegane@med.u-toyama.ac.jp

M. Shimizu
Department of Pediatrics, School of Medicine, Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa University, Kanazawa, Japan

H. Matsukura
Department of Pediatrics, Saiseikai Toyama Hospital, Toyama, Japan

Introduction

Wiskott-Aldrich syndrome (WAS) is clinically characterized by thrombocytopenia with small platelets, eczema, and humoral and cellular immunodeficiency, and it is caused by a mutation in the *WAS* gene and deficient expression of WAS protein (WASP) [1]. The molecular defect in *WAS* also results in X-linked thrombocytopenia (XLT), which is a clinically mild form of WAS [2]. Patients with WAS exhibit an increased incidence of autoimmune diseases, in which vasculitis, autoimmune hemolytic anemia and kidney disease are the most common manifestations [3]. Recently, a study involving a large cohort of patients with XLT revealed that autoimmune diseases are also frequently observed in XLT [4].

Kidney disease, the majority of which is IgA nephropathy, is found in 4–19 % and 5 % of patients with WAS [3, 5] and XLT [4], respectively. IgA nephropathy is a glomerulonephritis that is immunohistochemically characterized by mesangial proliferation with diffuse IgA deposition [6]. Multiple pathogeneses of IgA nephropathy, including impaired response to mucosal antigens, delayed clearance of immune complexes from the circulation and abnormal interaction with mesangial IgA receptors, have been reported [7]. In the above hypotheses, abnormal glycosylation of the IgA1 molecule plays a critical role [6, 7]. The level of poorly galactosylated IgA1 in serum was found to be increased in patients with IgA nephropathy [8]. Aberrant IgA had an increased tendency to self-aggregate and form immune complexes with IgG antibodies, and it is therefore more likely to be deposited in the mesangium [6]. In *WASP*-deficient mice, the increased production of aberrant IgA was strongly related to the development of IgA nephropathy [9].

The reason that aberrant IgA is produced in WAS/XLT remains unclear, but immunological dysfunction may play an important role [10–12]. Therefore, there is a possibility

that immune reconstitution improves IgA nephropathy. Our XLT patient demonstrated clinical and histochemical improvement in IgA nephropathy following bone marrow transplantation (BMT).

Material and Methods

Patient

The patient is a 19-year-old male, and he developed a subcutaneous hematoma at the age of 3 years, and was found to have thrombocytopenia. The family history showed that his maternal uncle had thrombocytopenia and IgA nephropathy, which resulted in end-stage renal failure at the age of 30 years. At the age of 6 years, the patient was diagnosed as having an XLT (G1487A).

Subsequently, he remained well, and his platelet count fluctuated between 50 and $100\times10^3/\mu L$. At the age of 8 years, the patient showed gross hematuria and proteinuria after an upper respiratory infection and was suspected to have glomerulonephritis (Table I). A percutaneous renal biopsy was performed, and he was diagnosed with IgA nephropathy [13]. He was treated with an angiotensin-converting enzyme inhibitor and low-dose prednisolone (PSL) for one year. He improved only partially, and the hematuria and proteinuria persisted. At the age of 14 years, he contracted an Epstein-Barr virus-negative atypical lymphoproliferative disorder and achieved remission after receiving 1 mg/kg per day of PSL.

Following the remission of lymphoproliferative disease, the patient underwent a BMT from an unrelated human leukocyte antigen (HLA)-matched donor. The pre-BMT conditioning regimen included busulfan (12.8 mg/kg), cyclophosphamide (200 mg/kg), rituximab (375 mg/m^2) and 3 Gy of total body irradiation. Tacrolimus (day -1 to 30, 0.01 mg/kg/day administered with continuous infusion, target serum levels of 10–15 ng/mL; from day 31 converted to oral at 0.03 mg/kg/day, target trough serum levels of 5 ng/mL), methotrexate (day 1, 15 mg/m^2; day 3, 10 mg/m^2) were used for graft-versus-host disease (GVHD) prophylaxis. Tacrolimus was tapered from 12 months after BMT and discontinued 20 months after BMT. PSL (0.2–0.5 mg/kg/day) was used for engraftment syndrome and GVHD for 8 months. The patient had complete chimerism soon after BMT. A second renal biopsy was performed 24 months after BMT (Table I).

Measurement of Lectin-Binding Serum IgA Levels

To examine the terminal galactosylation of IgA molecules, we used lectin-binding assays with Helix aspersa (HAA), which

Table I Laboratory findings of patient 1 before and after BMT

Test	At the first biopsy	1 year after low-dose PSL	Just before HSCT	At the second biopsy	Value
WBC	12,110	8,280	8,020	7,220	/μL
RBC	431	436	487	472	$\times10^4/\mu L$
Hemoglobin	13.9	12.4	14.5	14.5	g/dL
Hematocrit	39.1	35.4	41.6	42.4	%
Platelets	82	49	158	232	$\times10^3/\mu L$
BUN	9	10	14	7	mg/dL
Creatinine	0.5	0.5	0.8	0.6	mg/dL
IgG	613	916	899	1,060	mg/dL
IgA	264	315	261	131	mg/dL
IgM	23	18	116	267	mg/dL
CH50	34	NE	NE	NE	U/mL
C3	105	NE	NE	NE	mg/dL
C4	35	NE	NE	NE	mg/dL
Urinalysis					
Protein	2+	2+	2+	neg	
Occult blood	3+	+	+	neg	
RBC	100-150/HPF	10-19/HPF	10-19/HPF	neg	
WBC	5-10/HPF	5-10/HPF	neg	neg	
Hyaline cast	+	neg	neg	neg	
Granular cast	+	neg	1-4/WF	1-4/WF	

WBC white blood cells, *RBC* red blood cells, *BUN* blood urea nitrogen, *Ig* immunoglobulin, *CH50* complement activity, *NE* not examined, *C* complement, *HPF* high power field, *WF* whole field

recognizes terminal N-acetylgalactosamine (GalNAc) residues [14]. The ratio of the absorbance at 450 nm between IgA bound to HAA and total IgA (HAA/IgA) was calculated. The amount of galactose-deficient IgA in serum was estimated using the following formula: serum IgA concentration (C_{IgA})×HAA/IgA ratio.

Results

The first renal biopsy revealed that all of 28 glomeruli showed diffuse mesangial hypercellularity, and 4 glomeruli were completely scarred-in (Fig. 1a). Immunofluorescence studies showed IgA and IgG deposits were present, mainly in the mesangium (Fig. 1b and c). Electron microscopy showed a cluster of electron-dense deposits in the mesangium (Fig. 1g). The second renal biopsy showed that all 14 glomeruli were normal without mesangial proliferation, although 2 glomeruli showed mesangial sclerosis (Fig. 1d). IgA and IgG deposits significantly improved in immunofluorescent studies (Fig. 1e and f). Electron microscopy confirmed the disappearance of electron-dense deposits (Fig. 1h).

The IgA-dominant immune deposits in the mesangium and glomerular injury in the patient significantly improved after BMT. The amount of galactose-deficient IgA in his serum also markedly decreased after BMT (before BMT; 126.4 mg/dL, after BMT; 36.6 mg/dL) [14].

Discussion

This may be the first report to provide a detailed description of IgA nephropathy associated with WAS/XLT after BMT. The clinical course of the patient indicates that BMT can improve the IgA nephropathy associated with WAS/XLT. Although the detail was not described, a patient with IgA nephropathy and chronic myelogenous leukemia has been reported who had a remission not only of the leukemia but also of the IgA nephropathy after BMT [15]. In murine models of IgA nephropathy, IgA deposition in the glomerular lesion decreased after BMT from quiescent murine [16]. Conversely, BMT from the onset murine to quiescent murine resulted in IgA deposition in the glomerular lesion [17]. This study suggests that hematopoietic stem cells or their differentiated cells are related to IgA nephropathy. Recently, abnormalities of lymphocytes, which may be related to the production of aberrant IgA, have been reported [18–24]. B cells show abnormal activity of β-1,3-galactosyltransferase or α-2,6-sialyltransferase, which is the enzyme associated with glycosylation of the IgA1 molecule [18–20] and abnormal regulation of Toll-like receptors [21,

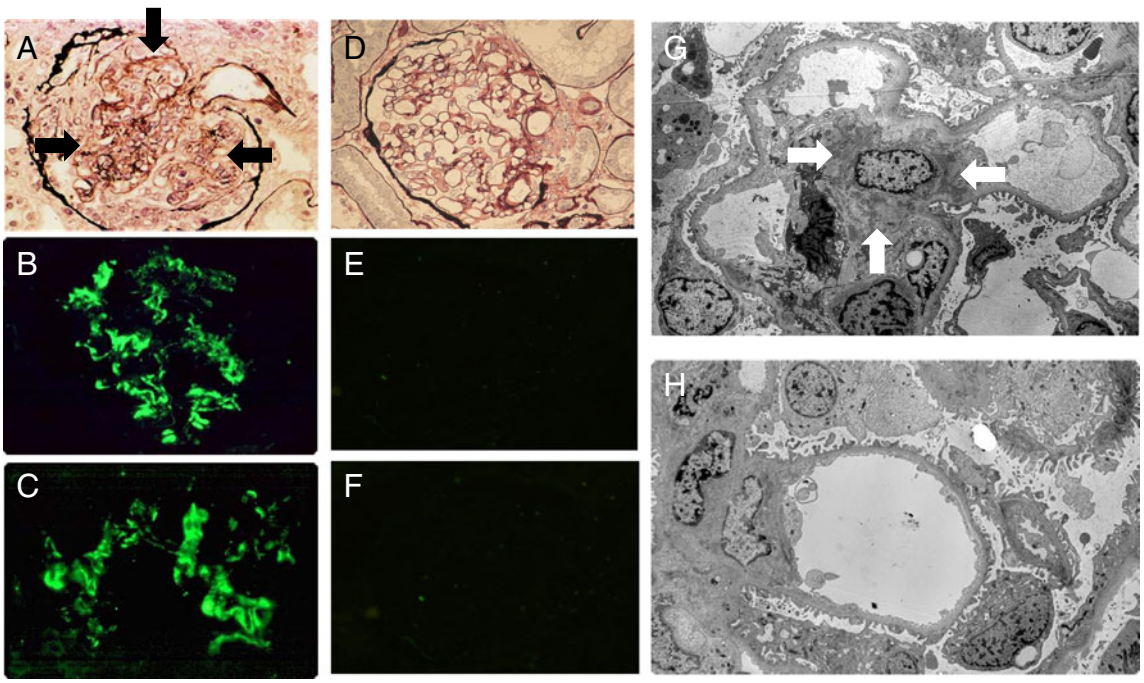

Fig. 1 Histological findings of renal biopsy samples from patient 1 before and after BMT. Histology of renal biopsy before (**a**, **b**, **c** and **g**) and after BMT (**d**, **e**, **f** and **h**). Periodic acid-methenamine-silver staining (**a** and **d**). Immunofluorescence studies of IgA (**b** and **e**) and IgG (**c** and **f**). Electron microscopy (**g** and **h**). Diffuse mesangial proliferative glomerulonephritis was observed (*arrows*) (**a**), and IgA and IgG deposits were observed in the mesangium (**b** and **c**) before BMT. Electron microscopy showed a cluster of electron-dense deposits in the mesangium (*arrows*) (**g**). Little proliferation of mesangium and cells was observed (**d**), and IgA, IgG and electron-dense deposits disappeared (**e**, **f** and **h**) after BMT

22]. T cells show abnormal secretion of IL-17 and T helper (Th) 2 cytokines in IgA nephropathy [19, 23].

The reason for the production of aberrant IgA and the development of IgA nephropathy in WAS/XLT remains unclear, but abnormalities of lymphocytes may play an important role. Consistent with previous reports, decreased levels of aberrant IgA and improvement of IgA nephropathy after BMT were observed in this patient. It has been reported that aberrant IgA and B cells which produce aberrant IgA increase age-dependently [9, 14], but there are no reports of mechanisms responsible for the aberrant glycosylation of IgA in WAS patients. However, impaired function of natural regulatory T cells and imbalance of Th1/Th2 cytokines, which are shown in IgA nephropathy, are also shown in WAS patients [10–12].

Histological improvement of IgA nephropathy might be caused by the clearance of mesangial IgA and the prevention of new IgA deposits. In renal transplantation, the deposits of mesangial IgA rapidly disappear after transplantation from donors with IgA nephropathy into recipients with non-IgA related disease [24, 25]. These observations seem to reflect a similar histological improvement. However, the precise mechanism remains unclear.

We suggest that hematopoietic stem cell transplantation (HSCT) for XLT patients can improve the long-term renal prognosis. HSCT at an early age is the treatment of choice for WAS patients [1]. However, therapeutic options for patients with XLT are controversial because of the excellent survival [4]. However, patients with XLT show a high rate of severe disease-related complications, and the prevalence of IgA nephropathy is 5 %; this condition occurs more frequently in Japanese patients, in whom the rate is 18 % [4]. Autoimmune diseases are significantly related to mixed/split chimerism for patients with WAS/XLT, suggesting that residual host lymphocytes or altered cytoskeleton can cause autoimmune diseases [26]. Therefore, adequate myeloablation and immunosuppression may be required.

Animal studies and the success in our patient support that HSCT may improve IgA nephropathy. Although in our patient, we cannot fully exclude the benefit of the myeloablative chemotherapy and post-transplant immunosuppressive GVHD-prophylaxis medications. Further studies are needed to define the role of the affected immune system in XLT and WAS in promoting IgA nephropathy, as well as, the role of immune reconstitution in improvement of the disease. Regardless, based on the supporting data from animal studies and our patient's beneficial result, HSCT should be considered for improving the clinical outcome in patients with XLT and IgA nephropathy.

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