Internal Medicine
The Japanese Society of Internal Medicine
doi: 10.2169/internalmedicine.9655-22
Intern Med 62: 269-273, 2023
http://internmed.jp

【 CASE REPORT 】

Chronic Graft-versus-host Disease-associated Membranous Nephropathy Following Bone Marrow Transplantation, Successfully Treated with Rituximab

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Abstract:
A 67-year-old woman who had undergone bone marrow transplantation 2 years previously for acute myeloid leukemia (AML) developed complications of chronic graft-versus-host disease (cGVHD). She thereafter also developed nephrotic syndrome, and membranous nephropathy (MN) was diagnosed by a renal biopsy. Although the causative antigens of the MN were not detected, immunofluorescence staining showed co-dominant deposition of immunoglobulins G2 and G3, a finding indicating secondary MN, thereby suggesting an association between MN and cGVHD. Rituximab treatment was initiated, and her nephrotic syndrome gradually improved without relapse of AML. Our present case suggests that rituximab is a safe and effective therapeutic option for cGVHD-associated MN.

Key words: bone marrow transplantation, graft-versus-host disease, hematopoietic stem cell transplantation, membranous nephropathy, rituximab

(Intern Med 62: 269-273, 2023)
(DOI: 10.2169/internalmedicine.9655-22)

Introduction

The incidence of kidney diseases in patients who have received hematopoietic stem cell transplantation (HSCT) is relatively low (1). However, nephrotic syndrome occurs in some patients, and membranous nephropathy (MN) is the most common cause, accounting for about two-thirds of affected patients (2), followed by minimal change disease (3).

MN has broadly been classified into 2 types: the idiopathic type, in which patients do not have an underlying disease; and the secondary type, which is associated with a causative systemic disease. MN can reportedly develop in association with the manifestation of chronic graft-versus-host disease (cGVHD), and this type of MN is regarded as the secondary type (4). A reduction in immunosuppressive therapy is considered to be a risk factor for the development of MN (5); however, its precise pathogenesis and effective therapeutic strategies have not yet been clarified. Although a number of causative antigens of MN have recently been identified (6), potential causative antigens of this type of MN remain unclear.

We herein report a patient who developed cGVHD-associated MN two years after receiving bone marrow transplantation (BMT) for refractory acute myeloid leukemia (AML). Although the causative antigens of MN in this patient could not be identified, clinical improvement in her nephrotic syndrome was achieved by rituximab treatment, without any signs of relapse of AML.

Case Report

A 67-year-old Japanese woman had undergone HLA-matched unrelated BMT 2 years previously for refractory AML that was not in remission using a conditioning regimen of fludarabine (180 mg/m^2), intravenous busulfan (6.4 mg/kg), and melphalan (80 mg/m^2). The patient subsequently received tacrolimus and short-term methotrexate as

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Received: February 28, 2022; Accepted: May 9, 2022; Advance Publication by J-STAGE: June 21, 2022
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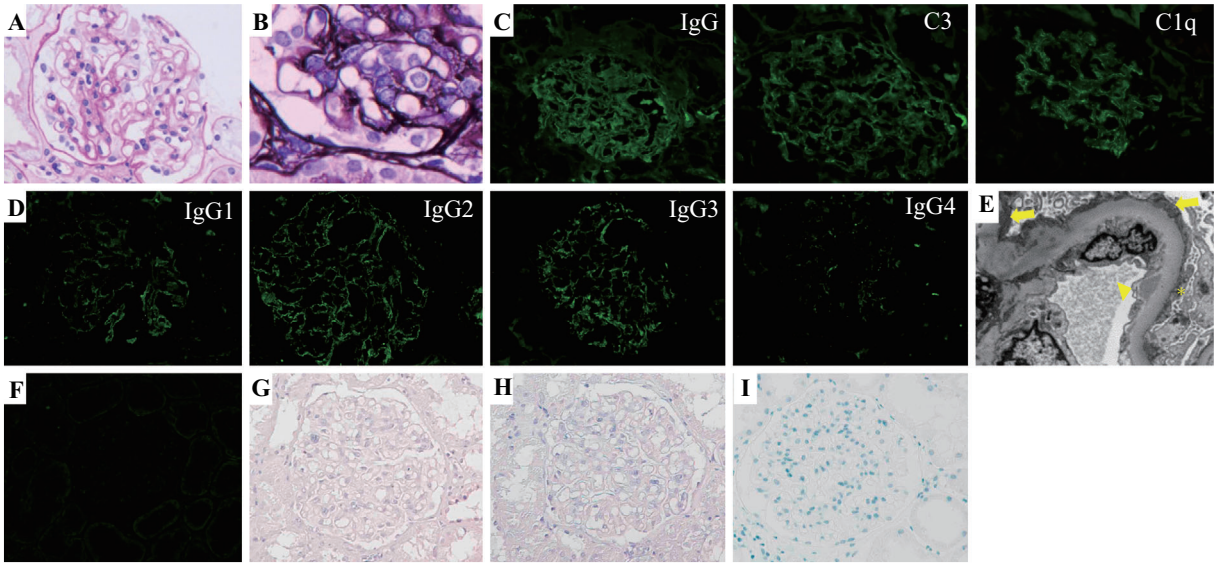

Figure 1. Histological features of the renal biopsy. (A) A light microscopy section showing a glomerulus with an almost normal appearance (periodic acid-Schiff stain). (B) Bubbly appearance of the glomerular capillary walls (periodic acid-methenamine-silver stain). (C) Granular deposition of immunoglobulin (Ig)G, as well as complement C3 and C1q on the glomerular capillary walls, shown by immunofluorescence (IF) staining. (D) Codominant deposition of IgG2 and IgG3 shown by IF staining of the IgG subclasses. IgG4 staining was negative. (E) Electron microscopy showing electron-dense deposits in the subepithelial (arrows) and subendothelial (arrowhead) areas and podocyte foot process effacement (asterisk). Spike formation of the glomerular basement membrane was ambiguous. IF staining for phospholipase A2 receptor (F; Sigma-Aldrich, St. Louis, USA) and immunoperoxidase staining for thrombospondin-type-1-domain-containing 7A (G; Sigma-Aldrich), neural epidermal growth factor-like 1 protein (H; Genetex, Irvine, USA), and hepatitis B virus surface antigen (I; Thermo Fisher Scientific, Waltham, USA) were all negative.

prophylaxes for GVHD and achieved complete hematological remission after BMT.

However, she subsequently developed stage 2 acute cutaneous GVHD and was treated with steroid ointment. Her skin rash did not improve, and she developed lichenoid changes in the skin and mouth, and keratoconjunctivitis sicca at three months after BMT and was diagnosed with moderate cGVHD (7). She started treatment with 20 mg prednisone, and her skin lesions improved, but her peripheral blood Wilms' tumor 1 (WT1)-mRNA level, which is a marker for the relapse of AML in the early phase (8), increased to 150 copies/μg RNA. Her immunosuppression was tapered as tolerated by her cGVHD symptoms, and her WT 1-mRNA levels decreased again to under the detection limit. While she was taking prednisone at 5 mg daily, she developed peripheral edema in her lower legs, which gradually worsened, and a urinalysis showed massive proteinuria. She was then referred to our hospital for a detailed examination.

Her vital signs were normal. She had peripheral edema, lichenoid lesions on her arms and legs, mild oral erythema, and dry eyes requiring lubricating eye drops 3 times a day. A urinalysis showed a urine protein level of 6.65 g/day but no hematuria (1-4 red blood cells/high-power field). The results of her blood analysis were as follows: white blood cell count, 6.3×10^3/μL; hemoglobin level, 12.1 g/dL; platelet count, 18.8×10^4/μL; serum creatinine, 0.60 mg/dL; blood urea nitrogen, 10.5 mg/dL; total protein/albumin, 5.1/1.8 g/dL; and immunoglobulin (Ig)G/IgA/IgM, 1,080/187/38 mg/dL. Analysis of serum IgG subclasses showed mildly decreased IgG2 at 178 mg/dL (reference range, 239-838 mg/dL), and normal levels of IgG1/IgG3/IgG4 (687/27.9/109 mg/dL; reference ranges, 351-962/8.5-140/4.5-117 mg/dL). Although serum protein electrophoresis showed a monoclonal IgG kappa spike, the serum free light-chain ratio was normal (1.63; reference range, 0.26-1.65), and urine protein electrophoresis was negative for Bence Jones protein. Hypocomplementemia was absent, and the antinuclear antibody titer was negative. Viral antibodies against hepatitis B surface and core antigens were positive, but hepatitis B surface antigen was negative, and serum hepatitis B virus DNA could not be detected. Anti-hepatitis C virus antibody was negative.

The renal biopsy yielded light microscopy sections of 22 glomeruli, in which 2 were globally sclerotic. There were no proliferative changes, and the glomerular capillary walls were not thickened (Fig. 1A). In contrast, a bubbly appearance was sparsely observed on the glomerular capillary walls (Fig. 1B). Tubulointerstitial changes were mild. Immunofluorescence (IF) staining showed granular deposition of IgG, and complements C3 and C1q on the glomerular capillary walls (Fig. 1C). IF staining for the IgG subclasses showed codominant deposition of IgG2 and IgG3, whereas

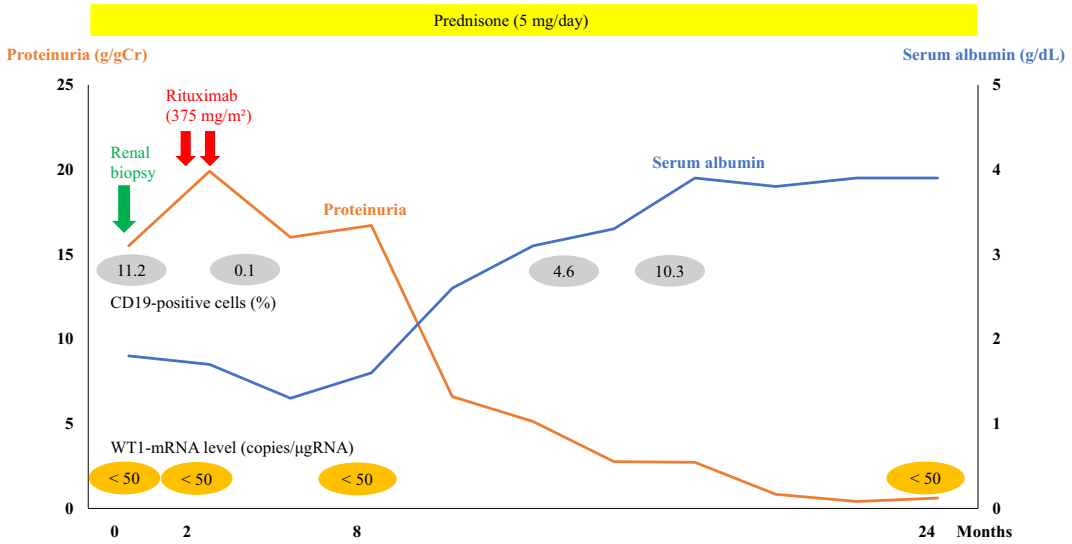

Figure 2. Clinical course of the patient. Rituximab was administered twice to the patient without changing the steroid dose (prednisone at 5 mg/day). The patient's nephrotic syndrome gradually improved despite the recovery of the CD19-positive cell number. The peripheral blood Wilms' tumor 1 (WT1)-mRNA level, which can detect the relapse of acute myeloid leukemia in the early phase, was periodically measured and confirmed to be within the undetectable range.

staining for IgG4 was negative (Fig. 1D). Subepithelial and subendothelial electron-dense deposits were observed on electron microscopy, but spike formation of the glomerular basement membrane was ambiguous (Fig. 1E). Based on these findings, the patient was diagnosed with stage I MN. Both serum anti-phospholipase A_2 receptor (PLA_2R) antibody assessed by the enzyme-linked immunosorbent assay and IF staining for PLA_2R were negative (Fig. 1F). Immunoperoxidase staining for thrombospondin-type-1-domain-containing 7A (THSD7A), neural epidermal growth factor-like 1 protein (NELL1), and hepatitis B virus surface antigen were also negative (Fig. 1G-I).

The renal pathological findings were compatible with secondary MN, but no causative diseases other than cGVHD were found, thereby strongly suggesting the possibility of MN secondary to cGVHD. The patient received rituximab twice (375 mg/m^2 per injection) without a change in steroid dose, as there were concerns regarding the possibility of a relapse of her AML. As shown in Fig. 2, her nephrotic syndrome gradually improved after the treatment without any signs of a relapse of AML; her peripheral blood WT1-mRNA level has remained within the undetectable range. Interestingly, although the proportion of her peripheral blood CD19-positive cells returned to normal, her urinary protein level and serum albumin level have been maintained at <1 g/day and >3 g/dL, respectively, during the 2-year follow-up period. Her skin cGVHD also resolved, whereas her ocular cGVHD has remained unchanged but in a stable condition.

Discussion

In the present patient, IF staining for the IgG subclasses showed codominant deposition of IgG2 and IgG3, without the deposition of IgG4. The deposition of complement C1q was also observed by IF staining. Although proliferative changes of the glomeruli were not observed, electron-dense deposits were found not only in the subepithelial areas but also in the subendothelial areas by electron microscopy. These pathological findings were consistent with secondary MN, which accounts for approximately 20% of patients with MN (9). In addition, our patient had complications from cGVHD. cGVHD occurring after allogeneic HSCT affects a variety of organs and causes substantial morbidity and mortality (7). A recent study reported that the skin, mouth, and eyes are commonly affected sites in Japanese patients as well as in Caucasian patients (10). Although the incidence of kidney diseases after HSCT is relatively low, MN reportedly occurs in association with cGVHD. A detailed examination of our present patient did not clarify any causative diseases other than cGVHD, so cGVHD-associated MN was strongly suggested.

We performed a literature review and summarized previously reported cases of cGVHD-associated MN in the post-PLA_2R era (Table) (2, 4, 11-14). The reported characteristics of cGVHD-associated MN include the following: 1) MN usually occurs about two years after HSCT (15), 2) IgG4- and/or IgG1-predominant deposition is observed despite a low rate of PLA_2R positivity (16), and 3) the renal outcome is generally favorable (17), at least in the short-term. Although these characteristics were mostly applicable to our present patient, the codominant deposition of IgG2 and IgG3 without the deposition of IgG4 was a unique finding that has not been reported to date. Although numerous causative antigens comprising the glomerular immune deposits of patients with MN have recently been identified, no

Table. Summary of Reported Cases of cGVHD-associated MN.

Patient	Age/ sex	Primary disease	Duration from HSCT to RBX (months)	MN stage	IF findings	IF IgG subclass	PLA_2R	Treatment of MN	Renal outcome	Reference
1	20/M	T-LBL	24	Stage I-II	IgG/A/M, C3/C4/C1q	Not described	Negative	PSL, CsA, CY	CR	12
3	44/M	AML	21	Stage I	IgG, C3/C4d	IgG1, 4	Positive	PSL, TAC	CR	4
2	35/M	ALL	18	Stage I	IgG	IgG1, 4	Not described	PSL, TAC	CR	4
4	48/M	NHL	15	Stage I	IgG	IgG1, 4	Negative	CsA	CR	4
5	48/F	DLBCL	32	Stage I	IgG, C3/C4/C1q	IgG1, 2, 3, 4	Negative	PSL, TAC	CR	4
6	50/M	MDS	40	Stage I	IgG	IgG1, 4	Not described	PSL, TAC	CR	4
8	60/M	LPL	9	Stage I	IgG	IgG1, 4	Negative	CsA	CR	2
7	54/M	MM	1	Stage I-II	IgG, C3	Not described	Negative	PSL	CR	13
9	62/M	MM	60	Stage I	IgG, C3/C4d	Not described	Negative	PSL, RTX	PR	14
10	46/M	ALL	91	Not described	IgG/A/M, C3/C4d/C1q	IgG1, 2, 3, 4	Positive	RTX	PR	11
11	62/M	AML	Not described	Not described	IgG, C3	Not described	Negative	PSL	PR	11
12	54/F	AML	36	Not described	IgG, C3	Not described	Negative	PSL	CR	11
13	69/F	AML	19	Not described	IgG, C3	IgG4	Negative	PSL, MMF	CR	11
14	70/F	AML	36	Not described	IgG, C3/C4d	Not described	Negative	PSL	CR	11
This case	67/F	AML	24	Stage I	IgG, C3, C1q	IgG2, 3	Negative	RTX	PR	-

M: male, F: female, T-LBL: T-cell lymphoblastic lymphoma, ALL: acute lymphoblastic leukemia, AML: acute myeloid leukemia, NHL: non-Hodgkin lymphoma, DLBCL: diffuse large B-cell lymphoma, MDS: myelodysplastic syndrome, MM: multiple myeloma, MMF: mycophenolate mofetil, LPL: lymphoplasmacytic lymphoma, HSCT: hematopoietic stem cell transplantation, RBX: renal biopsy, cGVHD: chronic graft versus host disease, MN: membranous nephropathy, IF: immunofluorescence, Ig: immunoglobulin, PLA_2R: phospholipase A2 receptor, PSL: prednisone, CsA: cyclosporine, CY: cyclophosphamide, TAC: tacrolimus, RTX: rituximab, CR: complete remission, PR: partial remission

corresponding antibodies predominantly of the IgG2 or IgG3 subclass have been identified to date (18). Indeed, renal biopsy tissue of the present patient was negative for immunostaining of these antigens, such as PLA_2R (19), THSD 7A (20), and NELL1 (21), although we did not evaluate other antigens, including α-enolase (22), which might have contributed to the pathogenesis of MN. The differences in the disease pathogenesis between reported patients with predominant glomerular deposition of IgG4 and/or IgG1 and cases like our patient without the deposition of IgG4 remain unclear and should be explored in a future study.

Thus, although the precise pathogenic mechanism underlying cGVHD-associated MN remains unclear, it is considered that alloreactive B cells, which produce antibodies against antigens in affected organs/tissues, play important roles (14). Therefore, rituximab, which targets B cells, may be a reasonable therapeutic option. Although there have been few reported cases to date of cGVHD-associated MN patients being treated with rituximab, rituximab is now widely used for the treatment of nephrotic syndrome. In addition, the efficacy and safety of a rituximab regimen comprising two infusions for the treatment of MN have recently been reported (23). We therefore administered two injections of rituximab as a treatment to our patient. In our patient, relapse of AML caused by an increase in steroid dose was a matter of concern, as her peripheral blood WT1-mRNA level had previously increased. The discontinuation of immunosuppressive therapy was desirable to enhance the graft-versus-leukemia effects (24); however, this was difficult because the patient developed MN. Rituximab administration was therefore performed without increasing the steroid dose and without the addition of cyclosporine, which improved the MN associated with cGVHD. Careful follow-up of this patient to monitor the relapse of nephrotic syndrome is crucial because her peripheral blood CD19-positive cells have already recovered; however, one possible cause of her condition may be the expansion of B cell clones that are not pathogenic to the development of MN after treatment with rituximab.

In conclusion, we reported a patient with cGVHD-associated MN in whom a favorable clinical outcome was achieved by rituximab treatment. Long-term follow-up of such patients will be required in the future to evaluate the efficacy and safety of rituximab for cGVHD-associated MN. In addition, further studies to identify as-yet-unknown causative antigens within the glomerular immune deposits of patients with this type of MN are required.

The authors state that they have no Conflict of Interest (COI).

Acknowledgement

We thank Ms. Helena Akiko Popiel at the Center for International Education and Research, Tokyo Medical University, for English-editing of the manuscript.

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