Kidney Medicine

Complement Receptor 1 Enhancement in Recurrent Membranous Nephropathy Following Kidney Transplantation: A Case Report

Noriyuki Kounoue, Hideyo Oguchi, Akinori Hashiguchi, Kazuho Honda, Dedong Kang, Tetuo Mikami, Naobumi Tochigi, Takeshi Kawamura, Yoshihiro Itabashi, Takashi Yonekura, Kei Sakurabayashi, and Ken Sakai

Membranous nephropathy (MN) recurs in some kidney allograft patients, and recurrence increases graft failure rates. We present a unique case of recurrent MN in first and second allografts showing glomerular capillary wall-positivity for complement receptor 1 (CR1) consistent with immunoglobulin G (IgG). A man in his late 20s developed MN and started hemodialysis. MN recurred and caused graft loss after the first transplantation and recurred again soon after the second transplantation. The IgG subclass staining was almost consistently negative for IgG4 and phospholipase A2 receptor (PLA2R)-staining was negative. Recurrent MN of unknown etiology was considered. Mass spectrometry demonstrated that CR1 had increased in the transplanted kidney biopsies. Immunohistochemistry and immunofluorescence studies demonstrated CR1 colocalized with IgG along glomerular capillaries in this case, whereas CR1 was localized in podocytes with no colocalization of IgG in a control case of PLA2R-associated MN. Correlative light and immunoelectron microscopy showed localization of CR1 at the interface between electron-dense deposits and podocytes. Collectively, this case demonstrated a unique enhancement and localization of CR1. MN with enhancement of CR1 has not been reported to date. CR1 may be a candidate causative antigen in this case of recurrent MN, although further study is needed to investigate the pathogenesis of CR1.

Complete author and article information provided before references.

Correspondence to H. Oguchi (hideyo.oguchi@med.toho-u.ac.jp)

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INTRODUCTION

A previous study demonstrated that the incidence of allograft loss due to recurrent glomerulonephritis is 8.4% over 10 years.[1] It has been reported that 18% of cases of membranous nephropathy (MN) recur 15 years after kidney transplantation, and recurrence increases overall and death-censored graft failure rates.[2]

Complement receptor 1 (CR1) is a membrane-bound glycoprotein in erythrocytes and various leukocytes.[3] CR1 has an affinity for C3b,[3] CR1 is also expressed by human glomerular podocytes and is located on the podocyte plasma membrane.[4] CR1 has been reported to play an important role as a complement regulator and immune adherence receptor using Chinese hamster ovarian cells expressing CR1, whereas the role of CR1 in podocytes remains unclear.[5] MN with enhancement of CR1 has not been reported in either native or allograft kidneys.

We introduce the first case of unique recurrent MN with CR1 enhancement after first and second kidney transplantations.

CASE REPORT

A man in his late 20s was diagnosed with MN. At the time of biopsy, his serum creatinine was 0.7 mg/dL and he had proteinuria of 5.6 g/g creatinine. Immunosuppressive therapy with steroids, cyclosporine, and mizoribine did not induce remission, and the patient subsequently began hemodialysis for kidney failure. Approximately 1 year after initiating hemodialysis, he underwent the first kidney transplantation, from his biologic sister. Ten months after transplantation, an allograft biopsy showed MN with proteinuria and elevated serum creatinine levels. Steroid pulse and apheresis therapy did not achieve improvement. The findings of MN were seen consecutively in an allograft biopsy series. We usually perform protocol biopsies 1 hour, 3 months, 1 year, 3 years, 5 years, 7 years, and 10 years after transplantation, and we performed more frequent follow-up biopsies because of persistent proteinuria and kidney function impairment in this patient. His allograft function continued to decline during 5 years of follow-up posttransplant, eventually necessitating reinitiation of maintenance hemodialysis. Two years later, the patient received a second kidney transplantation, from his wife. Three months after the second transplantation, an allograft protocol biopsy revealed MN, and the findings of MN continued in consecutive biopsies despite repeat steroid pulse, apheresis, and rituximab therapy.

Table 1 shows the consecutive histological results of light microscopy and immunofluorescence characteristics in the native kidney, and first and second allograft biopsies. After the first transplantation, glomerular basement membrane spike formation and immunofluorescence positivity for immunoglobulin G (IgG) demonstrated a diagnosis of MN 10 months after transplantation. MN was found continuously in the series of first allograft biopsies. We identified no specific immunofluorescence staining,

Table 1. Light Microscopy and Immunofluorescence Results of Consecutive Biopsies

Biopsies	Rejection	Spike	Bubbling	IgG	IgA	IgM	C3	C4	C1q	Fib	C4d	Diagnosis of MN
Native				+++	-	+	-		±	+		+
First allograft												
1 h	-	-	-									-
2 W	-	-	-	-	-	-	-		-	-		-
1 M	-	-	-	-	-	-	-			-		-
2 M	-	-	-	-	-	-	-			-		-
10 M	-	+	+	+	-	-	-		±	-		+
1 Y 5 M	-	+	+	+	-	-	-	-	-	-		+
1 Y 10 M	-	+	+	+	-	-	-	-	-	-		+
2 Y10 M	-	+	+	+	-	-	-	-	-	-		+
Second allograft												
1 h	-	-	-	-	-	±	-		-	-		-
3 M	-	-	-	++	+	±	-	-		-	+++	+
1 Y	-	-	+	++	+	+	-	+	±	±	+++	+
3 Y	-	+	+	++	++	+	+	++	±	+	+++	+
6 Y	-	+	+	++	++	+	±	++	+	++	++	+

Note: The empty boxes indicate no examination.
Abbreviations: W, weeks; M, months; Y, years; Fib, fibrinogen; MN, membranous nephropathy.

except for IgG. In the second allograft biopsies, MN findings developed with positive staining for IgG 3 months after transplantation. Glomerular basement membrane bubbling in light microscopy appeared 1 year after transplantation. In the second allograft biopsies, we observed changes in immunofluorescence. IgA, IgM, and C1q became positive 6 years after transplantation. In the native kidney and first allograft, C3 staining was negative, and in the second allograft, C3 staining was negative until 1 year after the second transplantation, positive at 3 years, and (±) 6 years after the second transplantation. No diagnosis of rejection was made after the first or second transplantation. Typical infections, malignancy, or autoimmune disease as causes of MN had not developed in the disease course.

IgG subclass, phospholipase A2 receptor (PLA2R), and thrombospondin type I domain containing 7A (THSD7A) staining, and electron microscopy results of biopsies are shown in Table 2. PLA2R and THSD7A were negative in the native and first allograft biopsies.

In terms of IgG subclasses, the native kidney was positive for IgG3 and negative for IgG4. No information about IgG1 or IgG2 was obtained. The first allograft biopsy 10 months after transplantation showed IgG1 (±), IgG2 (+), IgG3 (+), and IgG4 (−). The second allograft biopsy 3 months after transplantation demonstrated IgG1 (+++), IgG2 (+), IgG3 (++), and IgG4 (−). The last biopsy from the second allograft showed IgG4 (+) but with less intense staining than other subclasses, IgG1 (++), IgG2 (++), and IgG3 (++).

Investigations

We performed mass spectrometry using biopsies obtained at 10 months after the first transplantation and 6 years after the second transplantation. The results are shown in Fig S1. We detected complement factors and IgG1 in both allografts, which was compatible with MN. Previously reported antigens[6-11] (Item S1) were not detected. We found increased levels of complement receptor 1 (CR1; CD35) in allograft biopsies in our patient when compared with control tissues from another donor 1-hour biopsy and a native PLA2R-associated MN.

Immunohistochemistry demonstrated that CR1 was granularly positive along capillaries in our patient, and this finding was diffuse. CR1 was positive in the podocyte cytoplasm in control cases (Fig 1). The positive intensity along capillaries was stronger in the second allograft biopsy than that in the first allograft (Fig 1). Double immunofluorescence staining revealed colocalization of CR1 and IgG along capillaries in our patient (second allograft biopsy). A control case of PLA2R-associated MN showed no colocalization of CR1 and IgG (Fig 1).

The correlative light-electron microscopy (CLEM) results are shown in Figure 2. Positive staining for CR1 demonstrated localization at the interface between podocytes and electron-dense deposits (EDDs) in the second allograft in our patient. Some particles were present in EDDs. We performed the same analysis on tissue from the PLA2R-associated MN control case. The localization of positive PLA2R staining was also seen at the interface between podocytes and EDDs in the PLA2R-associated MN control case. The details of mass spectrometry, immunohistochemistry, immunofluorescence, and CLEM are described in the supplementary materials (Item S1 and Figure S2).

DISCUSSION

In this case, MN had occurred in the native kidney and had developed repeatedly in the first and second allografts. We diagnosed recurrent MN by kidney biopsy 10 months

Table 2. IgG subclass, PLA2R, and THSD7A Staining and Electron Microscopy Results of Consecutive Biopsies

Biopsies		IgG1	IgG2	IgG3	IgG4	PLA2R	THSD7A	EM stage
Native				+	-	-		II-III
First allograft								
1 h								
2 W								
1 M								-
2 M								I
10 M		±	+	+	-	-	-	I
1 Y 5 M								II
1 Y 10 M								
2 Y 10 M								II
Second allograft								
1 h								
3 M	+++	+	++	-				I
1 Y	++	-	+	-				II
3 Y								II
6 Y	++	++	++	+				II

Note: The empty boxes indicate no examination.
Abbreviations: PLA2R, phospholipase A2 receptor; THSD7A, thrombospondin type I domain containing 7A; EM, electron microscopy; W, weeks; M, months; Y, years.

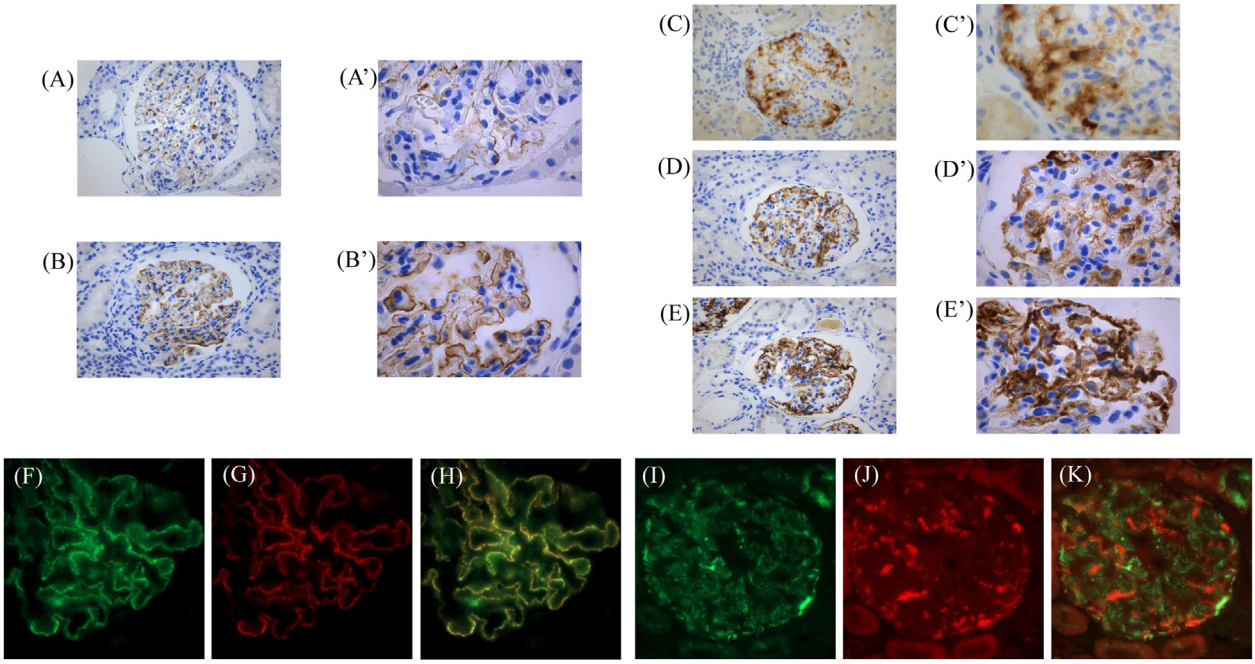

Figure 1. (A-E) Immunohistochemistry of CR1 (CD35). First allograft biopsy 10 months after transplantation (A) and second allograft biopsy 6 years after transplantation (B). Control cases are shown in (C-E). (C) PLA2R-associated MN in a native kidney, (D) minimal change disease in a native kidney, and (E) 1-hour postimplantation biopsy in another allograft. Original magnification, ×400. Extended magnified images are shown in (A'-E'). Biopsies in the present case of MN demonstrated granular positivity along capillaries (A, A', B, and B'). In control cases, no granular staining was observed along capillaries, and positive staining was seen only in podocytes (C-E). (F-K) IgG and CR1 (CD35) immunofluorescence. (F-H) Second allograft biopsy 6 years after transplantation in the present MN case. (F) IgG, (G) CR1, and (H) merged image. Granular positivity along capillaries was similarly observed in IgG and CR1 immunofluorescence staining, and colocalization was seen in the merged image. (I-K) PLA2R-associated MN control case. (I) IgG, (J) CR1, and (K) merged image. IgG and CR1 did not show colocalization. CR1, complement receptor 1; PLA2R, phospholipase A2 receptor; MN, membranous nephropathy.

posttransplant in the first allograft and 3 months posttransplant in the second allograft, both of which showed rapid development and poor treatment responses. In the native kidney and first and second allografts, immunofluorescence of IgG subclasses was mostly consistent, and C3 staining was mostly negative. Therefore, we considered

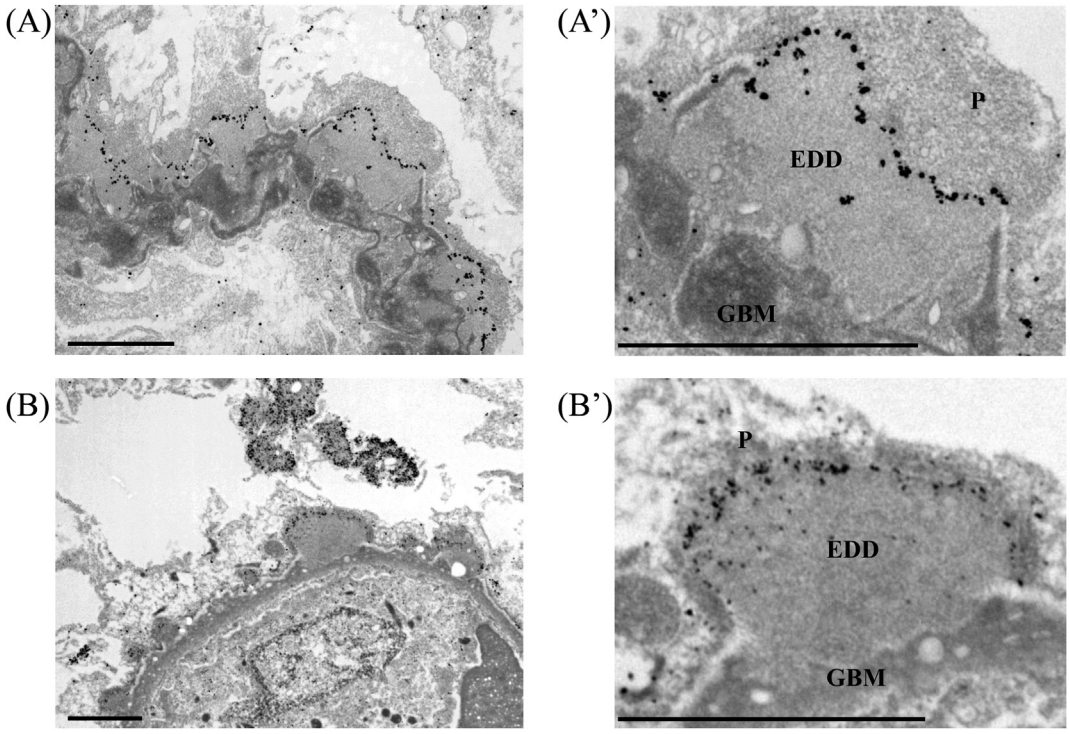

Figure 2. CLEM images. (A) CR1 (CD35) staining in the second allograft biopsy 6 years after transplantation in the present MN case. (B) PLA2R staining in a PLA2R-associated MN control case. (A' and B') Extended images. CR1 localized at the interface between podocytes and EDDs, and partially in the deposits. PLA2R had similar localization in PLA2R-associated MN. Scale bar indicates 10 μm. P, podocyte; EDDs, electron-dense deposits; GBM, glomerular basement membrane; CLEM, correlative light-electron microscopy; CR1, complement receptor 1; PLA2R, phospholipase A2 receptor; MN, membranous nephropathy.

recurrent MN. We did not identify other causes for MN, such as malignancy, autoimmune disease, and de novo MN from rejection. Additional investigations were performed to explore the etiology of MN.

Mass spectrometry results were compatible with MN, but we did not detect previously reported antigens associated with MN.[6-11] CR1 showed a significant increase in our patient when compared with controls, including another donor 1-hour biopsy and a native PLA2R-associated MN (Fig S1). Immunohistochemistry demonstrated CR1 expression in podocytes of control cases, whereas CR1 was localized in glomerular capillaries in our patient (Fig 1A-E). Double immunofluorescence staining revealed that CR1 and IgG were not colocalized in PLA2R-associated MN (Fig 1K), but CR1 and IgG were colocalized in the glomerular capillaries in our patient (Fig 1H). These results imply that CR1 translocated from the plasma membrane of podocytes to the glomerular basement side, which was consistent with IgG localization.

To examine CR1 localization in more detail, we performed CLEM. Unexpectedly, most CR1 was localized at the interface between podocytes and EDDs (Fig 2A). To the best of our knowledge, only 1 study has reported antigen and immunocomplex localization by immunoelectron microscopy in MN, and the membrane attack complex and hepatitis B e antigen were colocalized in EDDs in 5 of 6 cases of hepatitis B-associated MN cases.[12] In this study, we further examined PLA2R localization using CLEM in PLA2R-associated MN. CLEM showed that most PLA2R was expressed at the interface between podocytes and EDDs, which was similar to CR1, but we observed slightly more positive staining in EDDs when compared with CR1 (Fig 2B). Although we did not investigate the serum anti-CR1 antibodies or CR1 enhancement in the native kidney, these findings suggest that CR1 may be a novel candidate for MN-associated antigen.

It is interesting to note that most C3-negative staining was observed in a series of native and transplanted biopsies from our patient, although C3 was detected by mass spectrometry in first and second allografts (Table 1 and Fig S1). It is unclear why C3 was almost negative in the immunofluorescence of the biopsy series; however, CR1 antigen with CR1 localization change at the immune-complex interface might affect C3 antigenicity in immunofluorescence. A report hypothesized that CR1 has an important role in immunocomplex clearance, and immunocomplexes opsonized by complement are connected to CR1 on podocytes and caught in glomeruli to modify the course of inflammation.[5] Another report showed that CR1 is less abundant in primary MN cases when compared with controls in mass spectrometry and this change may activate complement pathways, causing marked proteinuria.[13] The

altered localization of the CR1 immune-complex interface may be an adaptation to the long-term deposition of immune complexes, possibly resulting in the increased detection of CR1 in our patient. Our patient had treatment resistance in the native kidney and after the first and second transplantations. This may be because CR1 function is inhibited by anti-CR1 antibodies that may be present.

This MN case leaves several unsolved issues. First, we did not clarify the pathogenesis of enhancement of CR1 in this case report. Second, we did not investigate whether MN with enhancement of CR1 is found in other unknown etiology MN cases besides this case. Third, we did not investigate serum anti-CR1 antibody in this case. Further study is needed regarding MN with enhancement of CR1, including investigation of serum anti-CR1 antibody.

In conclusion, we report a novel case of MN with enhancement of CR1 that recurred in the first and second allografts and presented with colocalization of CR1 and IgG along glomerular capillaries. CR1 could be a target antigen in the present case of recurrent MN, although further study is needed.

SUPPLEMENTARY MATERIALS

Supplementary File (PDF)

Figure S1: Mass spectrometry results.

Figure S2: CLEM immunofluorescence image.

Item S1: The details of mass spectrometry, immunohistochemistry, immunofluorescence and CLEM.

ARTICLE INFORMATION

Authors' Full Names and Academic Degrees: Noriyuki Kounoue, MD, Hideyo Oguchi, MD, PhD, Akinori Hashiguchi, MD, PhD, Kazuho Honda, MD, PhD, Dedong Kang, MD, PhD, Tetuo Mikami, MD, PhD, Naobumi Tochigi, MD, PhD, Takeshi Kawamura, MD, PhD, Yoshihiro Itabashi, MD, PhD, Takashi Yonekura, MD, Kei Sakurabayashi, MD, and Ken Sakai, MD, PhD

Authors' Affiliations: Department of Nephrology, Toho University Faculty of Medicine, Tokyo, Japan (NK, HO, TK, YI, TY, K Sakurabayashi, K Sakai); Department of Nephrology, Toho University Graduate School of Medicine, Tokyo, Japan (NK); Department of Pathology, Keio University School of Medicine, Tokyo, Japan (AH); and Department of Anatomy, Showa University School of Medicine, Tokyo, Japan (KH, DK); Department of Pathology, Toho University Faculty of Medicine, Tokyo, Japan (TM); and Department of Surgical Pathology, Toho University Faculty of Medicine, Tokyo, Japan (NT).

Address for Correspondence: Hideyo Oguchi, MD, PhD, 6-11-1, Omori-nishi, Ota-ku, Tokyo, Japan. Email: hideyo.oguchi@med.toho-u.ac.jp

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