Ultrastructural Studies of IgG4-related Kidney Disease

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Abstract

Objective Ultrastructural studies of IgG4-related kidney disease (IgG4-RKD) characterized by tubulointerstitial nephritis (TIN) are limited in previous reports due to the rarity of the condition. In the present report, we performed ultrastructural examinations and assessed the pathogenesis of this disease.

Patients Clinicopathological studies were conducted in eight patients diagnosed with IgG4-RKD. Routine light, immunofluorescence and electron microscopy examinations and immunohistochemical assessments of IgG4 were performed using renal biopsy samples.

Results Hypocomplementemia, positive anti-nuclear antibodies and eosinophilia were confirmed in more than half of the cases. Electron dense deposits (EDDs) were frequently found in the glomeruli and interstitium. The rate of deposition was 62.5% in both mesangial areas and Bowman’s capsule. EDDs were frequently detected on the tubular basement membrane (TBM) (87.5% of patients). The interstitium also contained EDDs on collagen fibers in 87.5% of the cases and on basement membrane-like materials in areas of fibrosis in 37.5% of the cases. The creatinine clearance levels were significantly lower in the patients with the latter pattern. Meanwhile, the rate of immunoglobulin and/or complement deposition on the TBM was observed in less than 37.5% of patients, and these findings were not entirely coincident with the cases of EDDs on the TBM.

Conclusion EDDs are frequently found in the glomeruli and interstitium in patients with IgG4-RKD; however, immunohistological studies do not provide evidence that IgG4-RKD involves TIN with immune complex nephropathy. The presence of interstitial EDDs may be related to the progression of interstitial fibrosis in the setting of IgG4-RKD.

Key words: plasma cell, electron dense deposit, immune complex

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Introduction

Recently much attention has been paid to the development of IgG4-related nephropathy and/or IgG4-related kidney disease (IgG4-RKD) characterized by tubulointerstitial nephritis (TIN), as these conditions present with unique clinical and histological findings. Initially, IgG4-RKD was reported to be an organ complication associated with autoimmune pancreatitis (AIP) (1, 2). Immunological disorders, such as hypocomplementemia, positive anti-nuclear antibodies and hypereosinophilia, are confirmed clinical features in more than half of patients with IgG4-related systemic diseases, including AIP and IgG4-RKD (3, 4). Based on this background, autoimmune and/or allergic disorders are suspected to underlie the pathogenic mechanisms of IgG4-RKD, although the detailed pathogenesis has not yet been clarified.

The Japanese Society of Nephrology (JSN) published diagnostic criteria for IgG4-RKD in 2011 (5). However, data regarding the electron microscopic findings of IgG4-RKD are limited at present. Some patients exhibit electron dense deposits (EDDs) on the glomerular basement membrane (GBM) and tubular basement membrane (TBM) (1, 2, 6, 7).

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The presence of ultrastructural abnormalities of the glomeruli and interstitium has not been thoroughly evaluated in patients with IgG4-RKD. We herein summarize the pathological findings of the glomeruli and interstitium observed on electron microscopy in eight cases of IgG4-RKD and discuss the pathogenesis of IgG4-RKD.

**Materials and Methods**

From 1999 to 2013, 6,342 patients underwent renal biopsies at Niigata University Medical and Dental Hospital, Kobe University Hospital and affiliated hospitals. Informed consent for the renal biopsy was provided by all patients prior to the procedure. Among the patients, eight were diagnosed with IgG4-RKD based on light microscopic observations and clinical findings according to the criteria of the JSN (5). Patients with collagen diseases and vasculitis were excluded, and no subjects met the criteria for systemic lupus erythematosus (SLE), Sjögren’s syndrome, anti-neutrophil cytoplasmic antibody (ANCA)-related vasculitis, rheumatoid arthritis or sarcoidosis.

Renal tissues were obtained via needle biopsy. All tissues were examined using routine light microscopy, direct immunofluorescence and electron microscopy. For light immunohistochemical staining, formalin-fixed, paraffin-embedded biopsy specimens were cut into 3-μm thick sections and immunostained with mouse monoclonal antibodies against human IgG4 (Zymed Laboratory, San Francisco, USA, or The Binding Site, Birmingham, UK).

Clinical data

All eight patients were men, with an average age of 71.3±9.6 (mean ± SD) years at the time of the renal biopsy (Table 1). Two cases involved complications of AIP. Renal imaging revealed multiple low density lesions in two cases on CT and diffuse kidney enlargement in six cases on enhanced CT.

The renal function was deteriorated in all patients. The mean creatinine clearance (Ccr) level was 41.2±21.8 mL/min/1.73 m² and the mean serum creatinine (S-Cr) level was 2.74±1.97 mg/dL at the time of biopsy. A significant amount of urinary protein, more than 0.2 g/day, was confirmed in six (75.0%) of the eight cases, and three (37.5%) of the eight patients had hypereosinophilia (Table 2). All patients demonstrated elevated serum IgG4 and IgG levels, with mean values of 3,424.5±1,024.8 mg/dL (cut off level

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**Table 1. Clinical and Laboratory Data in the Cases with IgG4 Related Nephropathy**

|    | age (years old) | sex | SBP (mmHg) | DBP (mmHg) | Ccr (mL/min/1.73m²) | S-Cr (mg/dL) | UP (g/day) | organ complication |
|----|-----------------|-----|------------|------------|---------------------|-------------|-----------|-------------------|
| case 1 | 68 | male | 118 | 68 | 36.3 | 1.37 | 0.1 |   |
| case 2 | 70 | male | 132 | 68 | 58 | 0.93 | 0.6 | AIP |
| case 3 | 75 | male | 122 | 70 | 66.9 | 1.23 | 0.3 |   |
| case 4 | 51 | male | 150 | 94 | 11.2 | 3.97 | 0.3 |   |
| case 5 | 83 | male | 112 | 60 | 44.4 | 4.72 | 0.7 |   |
| case 6 | 69 | male | 128 | 68 | 35.6 | 2.36 | 1.3 | AIP |
| case 7 | 78 | male | 167 | 70 | 11.8 | 6.17 | 1.4 |   |
| case 8 | 76 | male | 146 | 60 | 65 | 1.21 | 0.2 |   |
| mean | 71.3 | | 134.4 | 69.8 | 41.2 | 2.74 | 0.6 |   |
| SD | 9.6 | | 18.6 | 10.6 | 21.8 | 1.97 | 0.5 |   |

SBP: systolic blood pressure, DBP: diastolic blood pressure, Ccr: creatinine clearance, Cr: creatinine
AIP: autoimmune pancreatitis

**Table 2. Serological Data in the Cases with IgG4 Related Nephropathy**

| Eos | IgG4 | IgG | IgA | IgM | C3 | C4 | CH50 | ANF |
|----|-----|-----|-----|-----|----|----|------|-----|
| % | mg/dL | mg/dL | mg/dL | mg/dL | mg/dL | mg/dL | U/mL | times or index |
|----|-------|-------|-------|-------|-------|-------|-------|----------------|
| case 1 | 40 | 2,295 | 305 | 214 | 48 | '41 | '2< | '10 | '640 |
| case 2 | ND | 3,496 | 924 | 231 | 108 | 52 | '2< | '12.0 | '320 |
| case 3 | 6 | 5,380 | 623 | 273 | 87 | '41 | '2< | '14.1 | '100.1 |
| case 4 | 24.7 | 2,325 | 587 | 199 | 82 | 118 | 23 | 32 | ND |
| case 5 | 0 | 3,144 | 1,340 | 35 | 79 | 56 | '6 | '16 | '2,560 |
| case 6 | 7 | 4,001 | 1,860 | 145 | 99 | 55 | '2< | '10 | 80 |
| case 7 | ND | 3,935 | 670 | 113 | 74 | 57 | 28 | 27 | <40 |
| case 8 | *8 | 2,820 | 1,021 | 220 | 102 | 60 | 28 | 30 | ND |
| mean | 14 | 3,425 | 916 | 179 | 85 | 66 | 26 | 30 |   |
| SD | 15 | 1,025 | 494 | 77 | 19 | 25 | 3 | 2 |   |

Eos: eosinophil, ANF: anti nuclear antibody, ND: not done
* indicates abnormal data.
<135 mg/dL) and 916.3±494.2 mg/dL, respectively. Hypocomplementemia was noted in five (62.5%) patients, and anti-nuclear antibodies were positive in four (50.0%) patients. No cryoglobulin, M-peak proteins, myeloperoxidase (MPO)-ANCA or proteinase (PR3)-ANCA were detected in any of the patients.

**Histological findings**

TIN was a dominant histological feature in all cases. The cells comprising areas of massive infiltration primarily included lymphocytes and plasma cells, with occasional eosinophils. The distribution of infiltrating cells exhibited a specific pattern with a well-demarcated border between affected and unaffected areas (Fig. 1a), and the tubular basement membrane displayed thinning in the areas with massive cell infiltration. In the progressive fibrotic lesions, a characteristic ‘storiform’ pattern of fibrosis was observed surrounding nests of lymphocytes and/or plasma cells on periodic acid-methenamine staining of silver-stained preparations in all cases (Fig. 1b). Almost all glomeruli exhibited minor glomerular abnormalities, excluding two cases of membranous nephropathy.

Immunostaining of paraffin sections with anti-IgG4 antibodies showed infiltration of numerous IgG4-positive plasma cells into the interstitium (Fig. 1c). The number of IgG4-positive plasma cells >10/high power field (HPF) and ratio of IgG4-positive plasma cells/IgG-positive plasma cells exceeded 40% in all cases. Strong positive findings for IgG4 on the TBM and interstitium were confirmed in three (37.5%) of the eight cases. A small amount of IgG4-positive plasma cells was detected in both affected and relatively unaffected areas in the interstitium (Fig. 1d). IgG4 immunostaining also disclosed diffuse reactivity in the glomerular capillary wall in two cases of membranous nephropathy. In the other cases, the glomeruli showed no reaction to anti-IgG4 antibodies. No glomerular deposition of immunoglobulin or complement was observed on routine immunofluorescence examinations, excluding the two cases of membranous nephropathy. C3c deposition along the TBM was noted in three cases and IgG deposition along the TBM was seen in one case (Table 3).

EDDs were detected in various lesions on electron microscopy (Table 4). For example, subepithelial EDDs were observed in four (50.0%) patients, with segmental distribution in two cases and diffuse distribution in the two cases of membranous nephropathy (Fig. 2a) (Table 4). EDD deposi-
tion in mesangial areas was also seen in five (62.5%) of the eight cases; the mesangial EDDs were found in a scattered distribution in small amounts (Fig. 2b). Almost all of the EDDs had a homogenous appearance, although one case involved a mixed pattern of high and low electron dense materials (Fig. 2c). In addition, a small amount of subendothelial deposits was noticed in only one (12.5%) case. In contrast, EDD deposition in Bowman's capsules was detected in five (62.5%) of the eight cases (Fig. 2d), and a portion of the plasma cells contained a well-developed rough endoplasmic reticulum (Fig. 3a). Lymphocytes and eosinophils also infiltrated the interstitium (Fig. 3b). Furthermore, EDDs were visible on the TBM and collagen fibers themselves in 87.5% of the cases (Fig. 3b, c) and on basement membrane-like substances within fibrotic lesions in 37.5% of the cases (Fig. 3d).

### Relationships between the clinical data and the presence of EDDs

There were no clear relationships between the presence of serological disorders, such as hypocomplementemia and positive antinuclear factor (ANF), and the presence of EDDs on Bowman’s capsules, the TBM or interstitium. Cases 1 to 4 and 8 involved no deposition of IgG or C3c on the TBM, although these patients exhibited EDDs on the TBM. Three patients with EDDs on basement membrane-like substances within fibrotic interstitial lesions showed significantly lower Ccr levels, less than 36.0 mL/min/1.73 m², compared to that observed in the other cases (p<0.05; statistically significant difference according to the Wilcoxon test).

### Discussion

Previously reported organ disorders associated with IgG4-RKD include AIP as well as Mikulicz’s disease (8), sclerosing cholangitis (9) and retroperitoneal fibrosis (10). However, a few patients suffer from IgG4-RKD alone, without other types of organ involvement (3, 11, 12).

The pathological description of IgG4-RKD is limited to the findings of light microscopic and immunohistochemical assessments conducted in previous cross-sectional case studies. For example, Saeki (3), Kawano (13) and Yoshita (14) et al. each evaluated at least 20 patients with IgG4-RKD in their series using histological evaluations of light and immunohistochemical modalities without electron microscopy. To date, observations with electron microscopy have been restricted to limited case reports (1, 2, 6, 7, 15-17). Recently Yamaguchi et al. (18) comprehensively reported the ultrastructural findings of 10 cases. The authors found the deposition of EDDs on the TBM and interstitium in 100% and
Figure 2. Electron microscopy of the glomeruli. 2a: Subepithelial deposits exhibiting a segmental distribution. 2b: Mesangial deposits in paramesangial lesions. 2c: EDDs showing a mixed pattern of high and low electron dense materials. 2d: EDDs on Bowman’s capsules ×3,500.

Figure 3. Electron microscopy of the interstitium. 3a: Some plasma cells showed a tendency to assemble together. A well-developed rough endoplasmic reticulum was often observed ×3,500. 3b: EDDs on the TBM (star) and eosinophils in the interstitium (right upper cell) ×5,000. 3c: EDDs on collagen fibers (arrows) ×8,000. 3d: EDDs on basement membrane like-substances in the interstitium (arrowheads) ×8,000.
70% of cases, respectively. Meanwhile, Raissian et al. (19) documented the presence of EDDs on the TBM in 92.6% cases of IgG4-RKD. In the present study, the rate of EDD deposition was 87.5% on both the TBM and interstitium. Furthermore, we noticed two types of deposition in the interstitium: the presence of EDDs on collagen fibers themselves (87.5%) and the presence of EDDs on basement membrane-like materials within fibrotic lesions (37.5%).

Based on our experience, interstitial EDDs are seldom observed in other types of TIN, including cases of TIN induced by drugs or collagen diseases. The EDDs observed in patients with IgG4-RKD may therefore have affinity for the extracellular matrix of the basement membrane, as EDDs are frequently observed on the GBM (7, 18), Bowman’s capsule and TBM (1, 6, 7, 15, 18).

Cornell et al. (7) previously reported the existence of subepithelial EDDs in two of eight cases. Among our eight patients, four (50.0%) had subepithelial deposits, including subepithelial EDDs in two of eight cases. Among our eight capsule and TBM (1, 6, 7, 15, 18).

The immunopathogenic role of EDDs deposited on the TBM has not been fully clarified. The deposition of EDDs on the TBM is suspected to represent an immunopathological insult leading to the development of interstitial lesions in patients with lupus nephritis (20) and autoimmune TIN associated with anti-TBM antibodies (21). Additionally, EDDs are often detected on the TBM on kidney graft biopsies in patients without rejection (22, 23).

It should be carefully determined whether IgG4-RKD is an immune complex type of nephropathy. The immunofluorescence studies performed in the present series showed deposition of C3c or IgG on the TBM in only three (28.6%) cases, whereas most of the patients had EDDs on the TBM. In contrast, the immunohistochemical studies with anti-IgG4 antibodies did not show any remarkable reactions on the TBM on light microscopy. Similar results have been documented in previous articles (2, 4, 6, 11). These observations suggest that EDDs are not necessarily immune complexes involving IgG4. Subclass molecules of IgG exhibit distinct characteristics during immunological reactions. For example, it has been proven that IgG4 molecules do not have the ability to activate the complement cascade (24, 25). Detlefsen et al. (26) demonstrated the positive deposition of C3c, IgG4 and IgG on the basement membrane of the pancreatic duct in patients with AIP using immunofluorescence. The authors suspected that immune complex formation on the basement membrane triggered tissue destruction in cases of AIP. The pathological significance and structural components of EDDS remain unclear. Therefore, it is necessary to follow patients with renal involvement of IgG4-RKD by monitoring for immunoglobulin, complement and/or EDD deposition on the TBM and interstitium. In particular, in the present study, EDDs were found on basement membrane-like substances in fibrotic lesions in the patients with a significantly reduced renal function (p<0.05). This finding suggests the presence of a specific pathological process involving interstitial sclerotic changes in patients with IgG4-RKD. The clinical course of IgG4-RKD is usually responsive to steroids, although some patients exhibit deterioration of the renal function under steroid therapy (27).

In conclusion, we found that IgG4-RKD frequently presents with deposition of EDDs in multiple areas, including the glomeruli and interstitium. In particular, two types of EDD deposition in the renal interstitium were found in the present study: deposition on collagen fiber themselves and that on basement membrane-like substances in fibrotic lesions. However, our clinicopathological data did not demonstrate that EDD formation results from immune complex activation. Further investigation is therefore necessary in order to clarify the pathogenesis of IgG4-RKD in terms of both immunological and pathological perspectives.

Author’s disclosure of potential Conflicts of Interest (COI).
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