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An Unusual Case of Lysosomal Paraprotein Accumulation in Glomerular Endothelial Cells

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INTRODUCTION

Plasma cell dyscrasias (PCDs) are characterized by the excessive production of abnormal Igs, mainly free light chains (LCs) with nephrotoxic properties. Patients with PCDs develop a variety of renal diseases such as cast nephropathy (CN), amyloidosis, LC deposition disease (LCDD), and LC proximal tubulopathy. Cast nephopathy is characterized by intratubular cast formation of monoclonal LCs leading to renal dysfunction. LCDD and amyloidosis result from the deposition of monoclonal LCs, and LC proximal tubulopathy is caused by direct tubular damage due to monoclonal LCs and characterized by crystals or by an increased number of swollen lysosomes containing monoclonal LCs in proximal tubular cells. The presence of many enlarged lysosomes containing monoclonal LCs is documented most frequently in proximal tubular cells[1,2] and was reported in macrophages infiltrating the glomeruli in 1 patient with PCD[3]; however, such lysosomes are rarely observed in glomerular intrinsic cells. We herein report the case of a patient with multiple myeloma (MM) who developed a rare renal manifestation.

CASE PRESENTATION

An 80-year-old man with abnormal urinalysis findings was admitted to our hospital for renal biopsy. He had had vascular parkinsonism for several years, which was managed with regular medical checkups at the clinic. The patient had a history of hypertension, which was controlled by antihypertensive drugs. He had no history of urinary abnormalities or renal insufficiency for years before the renal biopsy. Two months before the renal biopsy, he was noted to have repeated proteinuria (2+ by dipstick urinalysis) and hematuria (1–4 red blood cells per high-power field) without an increase in serum creatinine level (0.8 mg/dl). He had frequent urination due to prostate hypertrophy and mild peripheral edema but was otherwise normal on physical examination. The possibility of glomerular injury could not be ruled out, and he was admitted to our hospital.

His laboratory test results were as follows: hematocrit, 29.6%; red blood cell count, 3.2 × 10^6/μl; white blood cell count, 6.8 × 10^3/μl; platelet count, 313 × 10^3/μl; total protein, 11.5 mg/dl (albumin [25.3%] and γ-globulin [57.4%] by serum protein electrophoresis); serum creatinine, 0.78 mg/dl; and blood urea nitrogen, 17 mg/dl. His β2-microglobulin level was increased at 6.7 mg/dl. The 24-hour urine specimen revealed the following: protein, 2.5 g (albumin, 47.1%; β-globulin, 35.7%; γ-globulin 1.2%; and A/G ratio, 0.9 by urine protein electrophoresis). The urinary β2-microglobulin level was 3370 μg/l. The IgG, IgA, and IgM levels were 9384 mg/dl, 96 mg/l, and 25 mg/dl, respectively. The serum free λ-LC level and free κ/λ ratio were 969 mg/dl and 0.026, respectively. The serum and urine immunofixation electrophoresis showed the presence of IgGλ monoclonal paraprotein. The serological tests were negative for antineutrophil cytoplasmic, antinuclear, and hepatitis B and C virus antibodies. No

cryoglobulins were detected. The bone marrow biopsy showed a hypocellular marrow with 90% plasma cells and fibrosis. The flow-cytometric analysis showed the proliferation of monoclonal λ plasma cells in the bone marrow. Therefore, the patient was diagnosed with IgGλ MM. Whether the proteinuria was due to renal injury related to PCD or to overflow proteinuria was unclear; therefore, a renal biopsy was performed.

Renal Biopsy Findings

On light microscopy, 6 of the 26 glomeruli in the biopsy sample were globally sclerosed, and striped fibrosis with mild arteriosclerosis was observed (Figure 1a). The remaining glomeruli showed no significant abnormalities except for eosinophilic granules within the cytoplasm of some of the glomerular endothelial cells in half of the glomeruli (Figure 1b and c). Luminal casts were not observed. By Masson trichrome staining, some bright red granules were observed in the proximal tubules, but crystal formation was not detected (Figure 1d). Congo red staining was negative.

Immunofluorescence examination of the paraffin-embedded tissue revealed granular positivity for IgG and IgG2 in the capillaries (Figure 1e and g) and in the cytoplasm of some of the proximal tubules (Figure 1f). The distribution of λ-LC was similar to those of IgG and IgG2. The granular positivity for λ-LC was detected inside and along the glomerular capillary walls (Figure 1h and i), tubular cytoplasm (Figure 1j), as well as in the inside of the peritubular capillaries (Figure 1j). The samples were negative for IgG1, IgG3, IgG4, IgA, IgM, C3, C4c, C1q, and k-LC (Figure 1k).

Immunohistochemical staining was performed for κ-LC, λ-LC, and lysosomes (Figure 1l–o). Granular staining for λ-LC was also present inside the glomerular capillaries (Figure 1m), but staining was negative for κ-LC (Figure 1l). The endothelial granules (Figure 1o, surrounded by circles) were positive for λ-LC and the lysosomal marker, which exhibited the same distribution on serial sections (Figure 1m and n).

The samples for electron microscopy contained only 1 glomerulus. Toluidine blue staining showed the accumulation of granules in some glomerular capillary lumens. The granules were attached to the capillary walls, suggesting that they were located in the endothelial cytoplasm (Figure 2a). The granular matter was not evident in other areas. Ultrastructurally, many swollen lysosomes were observed in the glomerular endothelial cells (Figure 2b) and, to a lesser extent, in the podocytes (Figure 2c) and the proximal tubular cells (Figure 2d). No electron-dense deposits or crystals were detected. The inside of the glomerular capillaries was filled with very fine, sand-like material (Figure 2b, inset).

Immunoelectron microscopic examination for κ-LC, λ-LC, and IgG2 revealed that the lysosomes contained particles against λ-LC in the endothelial cells (Figure 2e), podocytes (Figure 2g), proximal tubular cells (Figure 2h), and the sand-like material inside the glomerular capillaries (Figure 2i), all of which were positive for IgG2 as well (Figure 2j). No κ-LC was detected (Figure 2f). Based on these findings, the patient was diagnosed with monoclonal protein nephropathy with increased glomerular endothelial lysosomes containing λ-LC.

Follow-up

The patient was initiated on a combination treatment with bortezomib, dexamethasone, and cyclophosphamide, and achieved near-complete remission with reduced serum monoclonal protein level; his urinary protein became negative as well. The review of his medical records from the clinic revealed a spike in serum monoclonal antibody level that was already present 2 years before the renal biopsy in the absence of urinary abnormalities.

DISCUSSION

The current patient was diagnosed with IgGλ MM. Patients with MM develop a variety of renal diseases, most of which are diagnosed based on an algorithm that uses Congo red staining followed by immunofluorescence staining for monotypic LCs and heavy chains, and electron microscopy for the assessment of electron-dense deposits with or without organized structures (Table 1). However, the current case illustrates that the pathological diagnosis remains challenging and that immunoelectron microscopy is necessary as a definitive diagnostic approach, especially in patients exhibiting slight changes by light microscopy.

The current patient did not exhibit the pathological findings of CN or amyloidosis, both of which occur frequently in MM. LCDD is characterized by mesangial expansion or nodular sclerosis with monoclonal LC staining by immunofluorescence, and by punctate deposits along the glomerular and tubular basement membranes by electron microscopy. Although immunofluorescence staining or electron microscopy can detect LCDD at an earlier stage compared with light microscopy,[4] there were no findings suggesting LCDD in the present patient. Our case exhibited enlarged lysosomes containing λ-LC in the glomerular endothelial cells, which has been reported rarely except in patients with type I cryoglobulinemia.[5] Vankalakunti *et al*. reported a rare case of monoclonal gammopathy of renal significance, which had similarities with our case; their case presented monoclonal LC inclusions predominantly in the glomerular endothelial cells and

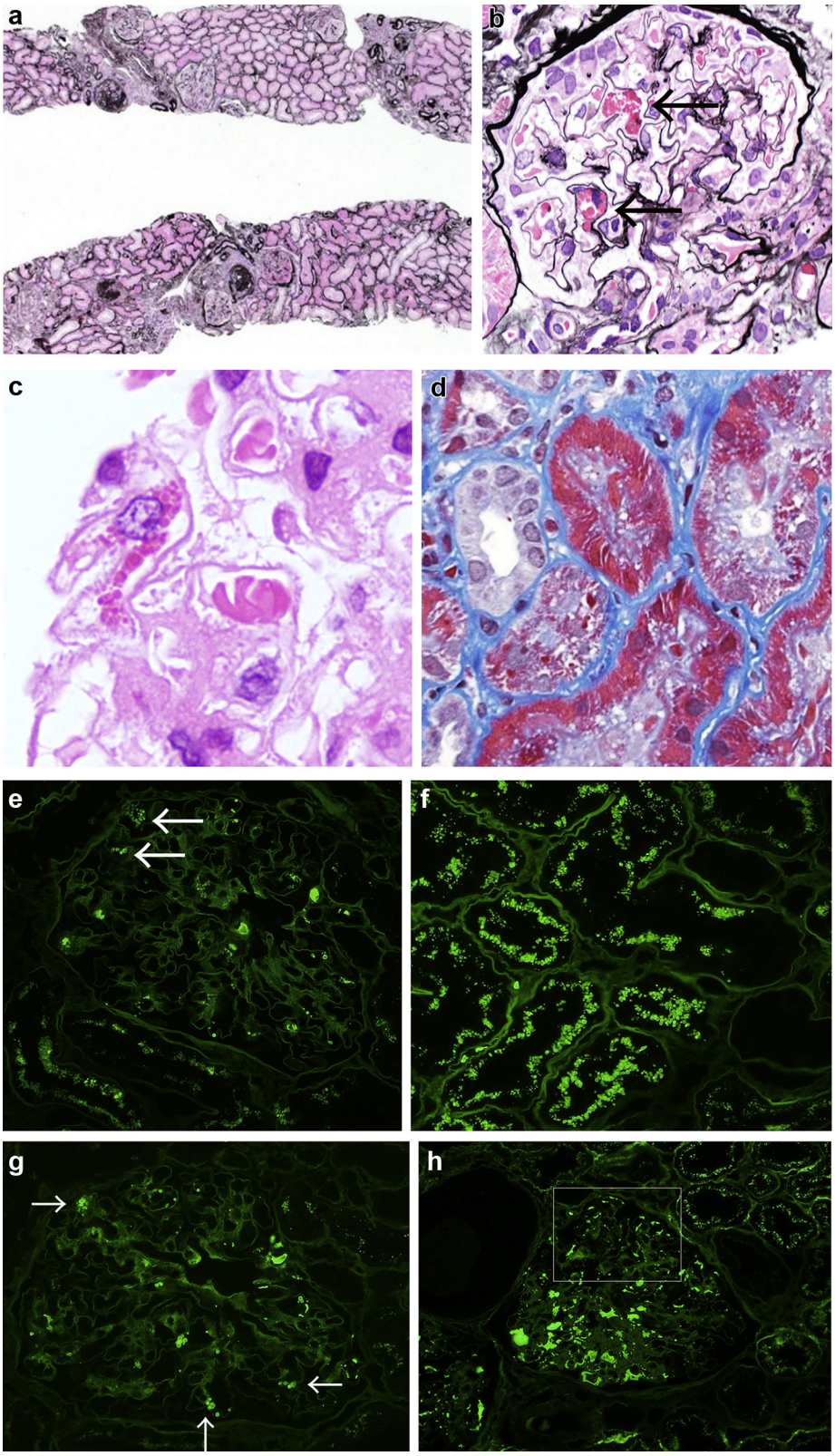

Figure 1. (a–d) Light microscopic, (e–k; e and f, IgG; g, IgG2; h–j, λ-light chain [LC]; k, k-LC) immunofluorescence, and (l–o) immunohistochemical microscopic findings. (a) Renal specimens containing several sclerotic glomeruli and striped fibrosis with mild arteriosclerosis. (b,c) No apparent abnormalities are noted except for eosinophilic granule-containing cytoplasms in some endothelial cells of some of the glomeruli (arrows). (d) Bright red granules are observed in the proximal tubules, but no crystal formation is detected (a,b, periodic acid–methenamine silver stain; c, hematoxylin–eosin stain; d, Masson trichrome stain; a, original magnification ×100; b,e, original magnification ×200; c, original magnification ×400). (e–j) Granular positivity for IgG is detected inside (e, arrows) the capillaries and (f) the tubular cytoplasm. Positivity for (g) IgG2 and (h–j) λ-LC is also observed in a localization that is similar to that of IgG. (Continued)

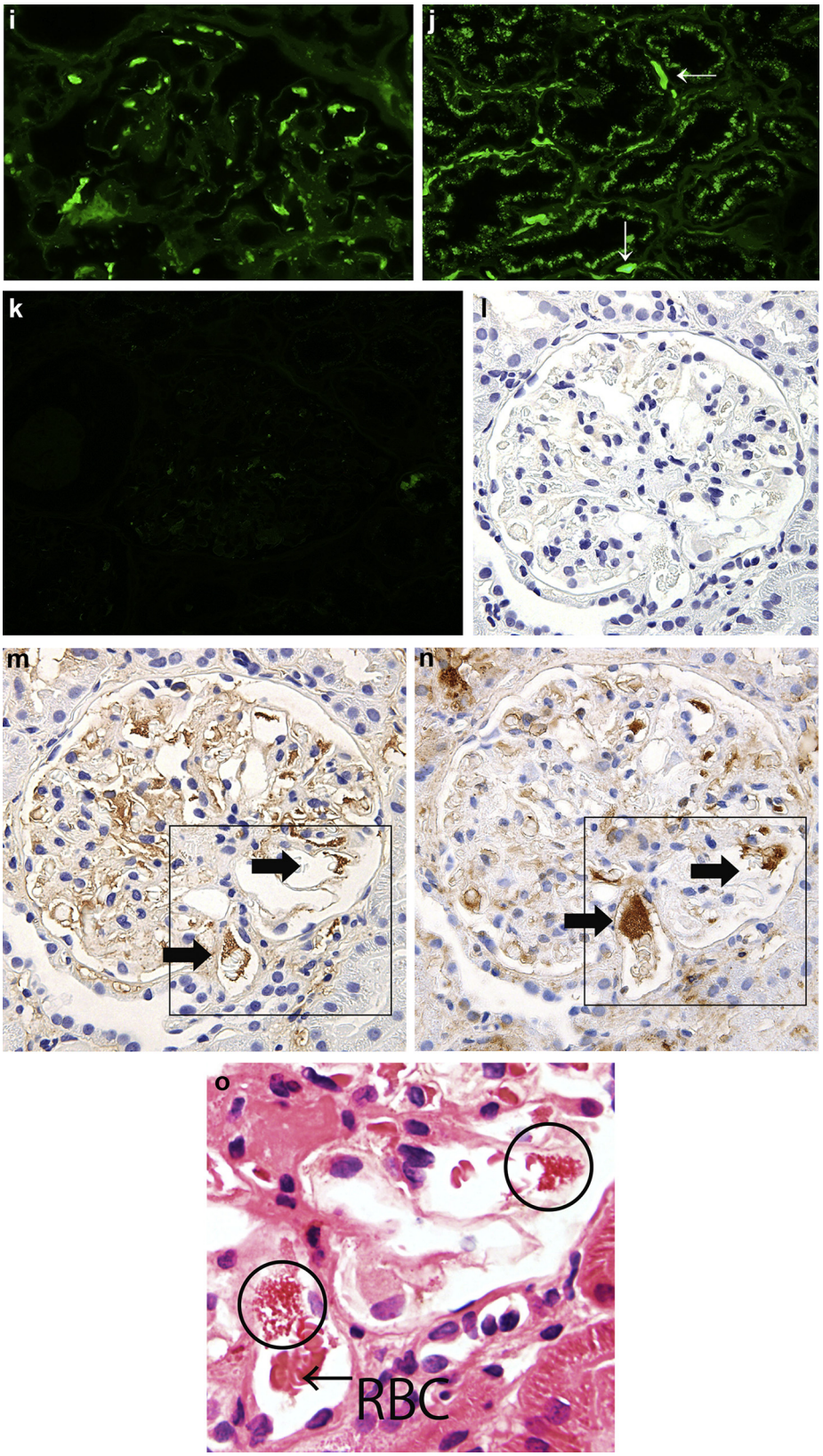

Figure 1. (continued) (i) At a high-power view of the square area in (h), λ-LC is observed along the inside of the capillary walls. (j, arrow) Strong positivity is also observed inside the peritubular capillaries. (k) No positivity is detected for k-LC (original magnification ×200). (l–o) Immunohistochemical and (o) hematoxylin–eosin staining of serial sections. (m, arrows) λ-LC immunostaining is positive inside the glomerular capillaries, which is colocalized with (n, arrows) lysosome immunostaining. (o) The enlarged image of the area within the squares in (m) and (n) (arrow points to red blood cell [RBC]). Endothelial granules in (o) (surrounded by circles) are positive for λ-LC and lysosomes (m and n, arrows), but negative for (l) κ-LC (l–o, original magnification ×400). Rabbit polyclonal anti-human lysosomal antibody (DAKO, A0099) was used.

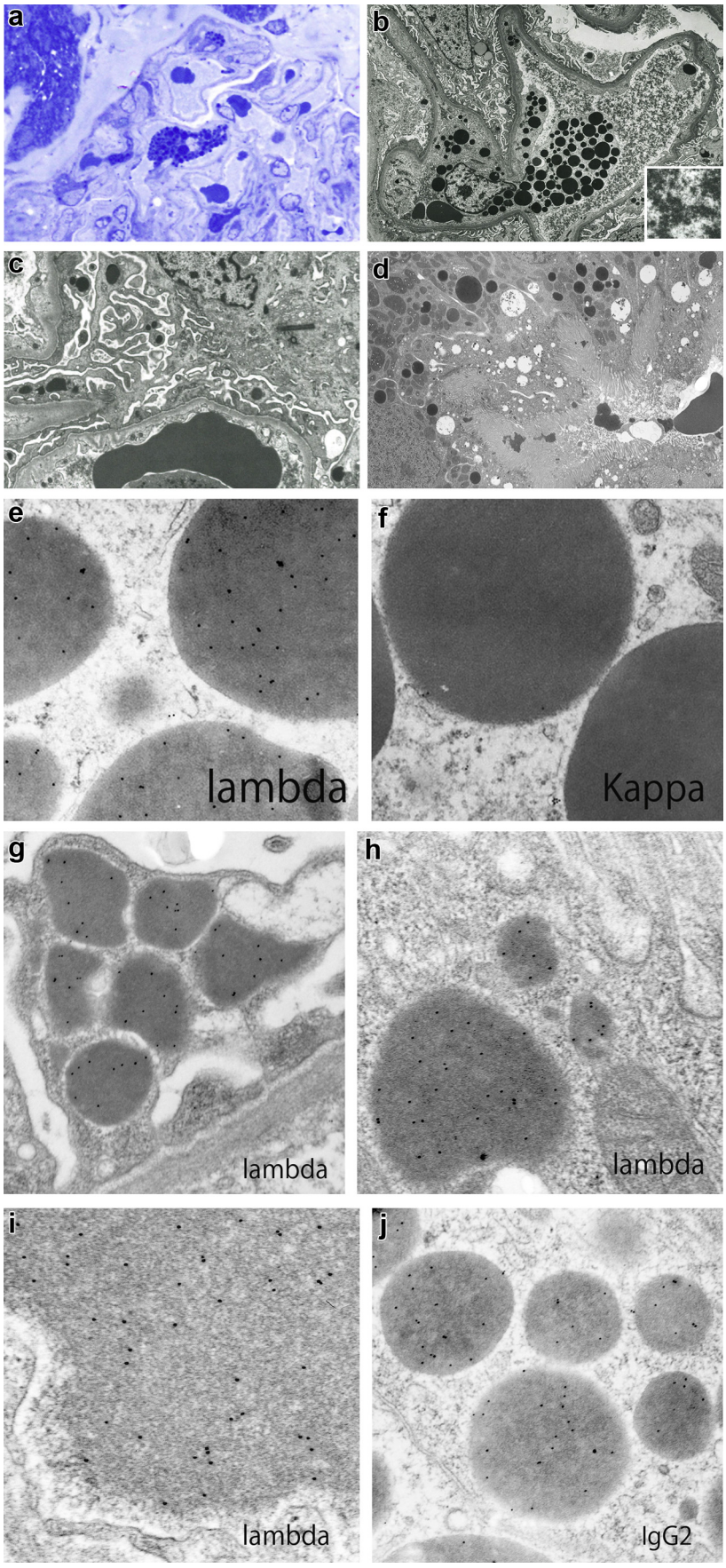

Figure 2. (a) Epon-embedded toluidine blue–stained section showing accumulation of granules inside the glomerular capillary lumen. Note that the granules are attached to the capillary walls in 2 locations (original magnification ×400). (b–d) Electron microscopic images. (e–j) (continued)

Table 1. Renal manifestations associated with plasma cell discrasias or monoclonal Ig/light chain deposits

Disease	Light microscopy	Location	Congo red	IF	EM
Glomerular disease					
Amyloidosis (AL/AH)	Amorphous eosinophilic materials	M, GBM, I, V	Positive	AL: Monotypic LC AH: Monotypic HC	Abundant fibrils (8–12 nm), random, nonperiodic, nonbranching
MIDD (LCDD, HCDD, and LHCDD)	Mesangial nodules	GBM, TBM, M	Negative	Monotypic LC alone, HC alone, or light and heavy chain	Nonorganized, nonfibrillary, powdery, punctate electron-dense deposits
PGNMID	Membranoproliferative/ endocapillary proliferative/ membranous/ mesangioprolifetrative patterns	M, GBM	Negative	Monotypic LC with single IgG subclass restriction (usually IgG3)	Nonorganized, nonfibrillary electron-dense deposits mimicking immune complexes
Immunotactoid glomerulopathy	Mesangioprolifetrative/ membranoproliferative/ membranous/endocapillary proliferative patterns	M, GBM	Negative	IgG often LC restriction	Microtubules (2–90 nm), random
Cryoglobulinemia (type I)	MPGN, pseudothrombi	M, GBM, vascular lumen (pseudothrombi)	Negative	Ig with LC restriction	Microtubular, fibrillary, or annular structures (10–30 nm), variable appearance
Crystalglobulinemia (variant of type I cryoglobulinemia)	Crystals within glomerular capillaries with intracapillary inflammation	Endothelium, proximal tubules	Negative	Monotypic Ig with LC restriction	Electon-dense rhomboid crystals
Diseases of tubulointerstitium					
Cast nephropathy	Intraluminal casts (eosinophilic and PAS negative) with giant cell reaction	Intratubules	Negative	Monotypic LC/HC	Crystalline, granular, or fibrillary casts
LCPT with crystals	Crystalline structures	Proximal tubule cytoplasms	Negative	Monotypic LC (usually κ)	Electron-dense crystalline structures
LCPT without crystals	Type 1: acute tubular injury Type 2: intracytoplasmic textured inclusions	Proximal tubule cytoplasms	Negative	Type 1: monotypic LC Type 2: monotypic LC (may need pronase digestion)	Type 1: increased lysosomes with irregular mottled appearance Type 2: fibrillary aggregates in cytoplasms
Present case					
Monoclonal protein nephropathy with increased glomerular endothelial lysosomes	Eosinophilic granules in glomerular endothelial cytoplasms	Glomerular endothelial cytoplasms (lesser extent in cytoplasms of podocyte and proximal tubules)	Negative	Monotypic LC with single IgG subclass restriction (present case is IgG2)	Increased swollen lysosomes with monoclonal LC detected by immunoelectron microscopy

EM, electron microscopy; GBM, glomerular basement membrane, I; HC, heavy chain; HCDD, monoclonal Ig heavy chain deposition disease; I, interstitium V; IF, immunofluorescence; LC, light chain; LCDD, monoclonal Ig light chain deposition disease; LCPT, light chain proximal tubulopathy; LHCDD, monoclonal Ig light and heavy chain deposition disease; M, mesangium; MIDD, monoclonal Ig deposition disease; PAS, periodic acid–Schiff; PGNMID, proliferative glomerulonephritis with monoclonal Ig deposition, vessel wall; TBM, tubular basement membrane.

showed a good response to bortezomib.[6] However, their case demonstrated heavy proteinuria with renal insufficiency, and their renal biopsy revealed a membranoproliferative glomerulonephritis with crystalloid formation in glomerular endothelial cells,[6] which were different from ours. Although considered an atypical finding in MM, this presentation was considered to be associated with MM in the current patient because the urinary abnormalities improved after the treatment for MM.

The current patient had a high serum IgG level, and the immunoelectron microscopy showed that the sand-like material in the glomerular capillaries was positive for IgG2 and λ-LC. The serum IgG concentration is regulated by IgG with the Fc receptor (FcRn). After monoclonal and normal Ig are internalized by the endothelial endosomes via pinocytosis, IgG that binds to FcRn traffics to the cell surface and is exocytosed into the circulation, whereas unbound proteins are degraded in the lysosomes of endothelial cells[7] and then delivered to the opposite site of the cells to be exocytosed to the intercellular space.[8,9] Podocytes take up albumin and IgG into the lysosomes for degradation and consequent transcytosis to the urinary space.[9,S1]

Figure 2. (Continued) Immunoelectron microscopic images. (b) Electron micrograph shows glomerular endothelial cells containing abundant lysosomes (original magnification ×4000). (Inset) High-power photomicrograph showing the sand-like material inside the peripheral capillaries (original magnification ×8000). (c,d) Podocytes and proximal tubular cells containing swollen lysosomes (c, original magnification ×3000; d, original magnification ×2000). (e–i) Immunoelectron microscopy shows the presence of λ-LC in the lysosomes of the (e) endothelial cells, (g) podocytes, and (h) proximal tubular cells as well as (i) the sand-like material inside the peripheral capillaries, but (f) κ-LC is not detected (original magnification ×7000). (j) Immunoelectron micrograph showing IgG2 in the lysosomes of the endothelial cells (original magnification ×7000).

Table 2. Teaching points

1. The present case showed mild proteinuria without renal insufficiency as the presenting symptom of IgGλ MM.
2. Endothelial granules in some glomeruli are the only pathological change detected by light microscopy.
3. Immunoelectron microscopy revealed that those granules were enlarged lysosomes containing IgG2λ.
4. Careful light microscopic and ultrastructural examination is necessary in the diagnosis of MM.

MM, multiple myeloma.

The current patient exhibited enlarged lysosomes containing λ-LC in the glomerular endothelial cells, podocytes, and proximal tubular cells. LC proximal tubulopathy is characterized by crystal formation inside the cytoplasm of proximal tubular cells. However, if the endocytosed LCs do not have innate physicochemical properties that resist proteolysis and promote self-aggregation, crystal formation will not occur,[S2–S4] and enlarged lysosomes filled with monoclonal LCs accumulate in the proximal tubular cells.[S5] These lysosomes are unable to release their enzymes; therefore, the reabsorptive functions of the proximal tubular cells eventually cease, leading to Fanconi syndrome.[S5] Apart from renal intrinsic cells, accumulation of swollen lysosomes with monoclonal LCs is detected in macrophages infiltrating to the glomeruli in MM,[3] in which case the phagocytic function of macrophages may contribute to the removal of LCs to promote renal repair.[3]

Although the current patient did not exhibit λ-LC in the mesangial cells or the matrix, an *in vivo* study on mice that were injected with free LCs purified from the urine of patients with amyloid LC-amyloidosis demonstrated the physiological mechanism of amyloid LC deposition in the mesangium.[S6] The authors showed the phenotypic transformation of the mesangial cells to CD68-positive macrophages and the increase in the number of lysosomes in the mesangial cells following the administration of the free LCs, which were then endocytosed into the lysosomes through the caveolae; the lysosomes were then abutted on the mesangial cell membranes and extruded the amyloid-LCs into the extracellular matrix.[S6]

The mechanism of proteinuria in the current patient is unclear. One possibility is lysosomal dysfunction in the glomerular endothelium, which might have impaired the barrier function of the glomerular capillaries via a reduction in the negative charge of the glomerular basement membrane and glycocalyx that coats the endothelium and prevents the passage of large proteins. In Fabry disease, a lysosomal storage disease that leads to lysosomal dysfunction, glomerular endothelial fenestration is decreased,[S7] which accompanies a reduction in nitric oxygen bioavailability in the endothelium.[S8] Another hypothesis is that lysosomal dysfunction might have led to an increase in the number of autophagosomes with the subsequent dysregulation of autophagy. Deficiency in autophagy in murine glomerular endothelial cells leads to capillary rarefaction,[S9] which may subsequently result in proteinuria.

As summarized in Table 2, this is a case of mild proteinuria without renal insufficiency as the presenting symptom of IgGλ MM. Endothelial granules in some glomeruli are the only pathological change detected by light microscopy, which is a rare presentation in MM. Immunoelectron microscopy revealed that those granules were enlarged lysosomes containing IgG2λ. The current case emphasizes the utility of careful light microscopic and ultrastructural examination in the diagnosis of MM, which should be elucidated in additional case reports.

DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary References.

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