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Single Case

A Case of Mesangial Proliferative Nephritis Caused by Slow Cryoglobulin

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Keywords
Slow cryoglobulin · Cryoglobulin · Membranous glomerulonephritis

Abstract
The patient was a woman in her 60s. She was found to have proteinuria on a health checkup. She did not have any particular subjective symptoms, and no definitive diagnosis was made, despite serological findings indicative of immune abnormalities. A renal biopsy was performed. Light microscopy of renal tissue section revealed mesangial proliferative nephritis. Electron microscopic findings included electron-dense deposits and fibrillar/tubular structures with a diameter of 20–30 nm. These findings suggested the presence of cryoglobulin (CG), but CG was not detected in qualitative or quantitative hematologic tests. Thus, the serum samples were stored at 37°C for a long period of time and then cooled to 4°C. When the obtained precipitates were examined, CG was successfully detected. CG that precipitates only after a long period of time is referred to as slow cryoglobulin (sCG), and sCG is extremely rare. The present case is the first documented case, to our knowledge, of renal disorders caused by sCG. It should be noted that there are some cases in which it takes much time for CG to precipitate. Thus, when CG cannot be detected, it is necessary to spend much time to determine whether CG precipitates.

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Introduction

Cryoglobulins (CGs) are cryoprecipitates that precipitate at 37°C or lower and redissolve on warming. They are comprised of immunoglobulins alone or together with complement components. According to the Brouet classification based on the clonality, which is now commonly used, cryoglobulinemia can be classified as type I, II, or III or mixed type [1]. Many patients with cryoglobulinemia are asymptomatic, and symptomatic cases are referred to as CG syndrome or CG vasculitis. There are no established diagnostic criteria. While cryoglobulinemia is comprehensively diagnosed based on clinical symptoms, laboratory findings, and pathological findings, detecting CG in blood or tissues is essential for the diagnosis. It takes much time for some CGs to precipitate, and they are referred to as slow CG (sCG) [2]. Although only limited reports are available on sCG, the pathology associated with sCG is not as yet understood. Moreover, the fact that sCG does not precipitate within the regular processing time frame is a plausible explanation for the fact that many cases are overlooked. When histological examination by electron microscopy reveals CG-like substances, cryoglobulinemia should be considered. The CARE Checklist has been completed by the authors for this case report, attached as supplementary material (for all online suppl. material, see https://doi.org/10.1159/000531736).

Case Presentation

Patient was a woman in the 60s whose Chief concern was renal disorders and who had medical history of acute hepatitis B (cured), retinal detachment, and hypertension and family history of rheumatoid arthritis in her mother with no life history of drinking, smoking, or allergy or history of present illness.

A woman in her 60s since the patient was found to have proteinuria on a health checkup, she presented to our department in year X. She tested positive for antinuclear antibody and presented with pancytopenia, proteinuria, and hypocomplementemia, so that systemic lupus erythematosus (SLE) was suspected. She was admitted to our hospital for detailed examination and treatment in the following year.

Physical findings on admission: No particular abnormal findings No rash was noted. No Raynaud's phenomenon was noted. No edema was noted. The body temperature was 36.7°C. Blood pressure was 143/74 mm Hg.

Laboratory findings on admission are shown in Table 1. Pancytopenia and hypocomplementemia were detected. The patient tested positive for antinuclear antibody. Her rheumatoid factor (RF) level was high. However, she tested negative for other various antibodies including anti-double-stranded DNA immunoglobulin (Ig) G antibody. She tested negative for CG by both qualitative and quantitative tests. She exhibited a pattern of previous hepatitis B virus infection. Urinalysis showed positive for protein and occult blood. The patient tested negative for hepatitis C antibodies and was free of other infections on screening tests. Urinary findings included slightly positive urinary protein and urinary occult blood. Urine sediment showed no abnormal findings.

Figures 1–5 shows the pathological findings of renal biopsy samples. The samples collected from the cortices of 25 glomeruli showed marked lobulation. Endocapillary cell proliferation, mesangial cell proliferation, and mesangial matrix proliferation were diffusely and globally observed. Hyaline thrombi were scattered. Wire loops-like lesions were also observed. The loop wall was diffusely and globally reduplicated. Deposits were also observed under the endothelium. Immunofluorescence (IF) test showed diffuse global deposition of full house immune complexes predominantly in the loop. Staining results were negative for

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Table 1. Laboratory findings of examinations on admission

WBC	2,000	/μL
RBC	413	$\times 10^4$/μL
Hb	10.2	g/dL
Ht	30.0	%
Plt	16.1	$\times 10^4$/μL
TP	6.1	g/dL
Alb	4, 0.1	g/dL
AST	27	IU/l
ALT	13	IU/l
LDH	297	IU/l
γGTP	12	IU/l
UN	22	mg/dL
Cr	0.9	mg/dL
UA	5.7	mg/dL
Na	143	mEq/l
K	4.3	mEq/l
TC	190	mg/dL
TG	74	mg/dL
CRP	<0.04	mg/dL
IgG	672	mg/dL
IgA	112	mg/dL
IgM	134	mg/dL
C3	54	mg/dL
C4	3	mg/dL
CH50	24.2	mg/dL
ASO	28	IU/mL
ANA	×160	
RF	1,028	IU/mL
IgG RF	0.2	IU/mL
MMP-3	29.0	ng/mL
Anti-β_2GPI	(−)	
Anti-ss-DNA	(−)	
Anti-ds-DNA IgG	(−)	
Anti-Sm	(−)	
PR3-ANCA	(−)	
MPO-ANCA	(−)	
Cryoglobulin	(−)	
Lupus anticoagulant	(−)	
Anti-thyroglobulin	(−)	
Anti-TPO	(−)	
PT-INR	0.96	

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Table 1 (continued)

APTT	23.2	sec
Urinalysis		
Protein	(+)	
Blood	(+)	
U-TP/Cr	0.6	g/g/Cr
Ccr	73.9	mL/min

hepatitis B surface antigen, hepatitis Be antigen, and hepatitis B core antigen. When the electron-dense deposit (EDD) area was magnified by 20,000 times by electron microscopy, fibrillar, or tubular structures with a diameter of 20–30 nm were observed.

Thus, the light microscopic findings indicate proliferative nephritis. If the patient had SLE, these findings would indicate that she had class 4 lupus nephritis. However, EDD shown by electron microscopy was greatly different from that observed in SLE.

Clinically, the patient did not meet the diagnostic criteria for SLE based on the 2019 ACR/EURA (seven criteria not met, including decreased WBC and complement). According to the 2012 SLICC classification, the patient did not meet three criteria, including a clinical criterion of decreased WBC and immunological criteria of antinuclear antibodies and hypocomplementemia. However, if the renal pathological diagnosis is lupus nephritis, the diagnostic criteria would be met, indicating the importance of renal pathology. Although the light microscopic and IF findings were suggestive of lupus nephritis, as described above, the electron microscopic findings were completely different from those of SLE. Hence, we concluded that the diagnostic criteria for SLE were not met. Nevertheless, findings, such as 20-nm fine fiber structures, are suggestive of CG.

Furthermore, no abnormalities were detected in the gastrointestinal tract or on imaging studies, and bone marrow examination showed no chromosomal abnormalities. Thus, there were no particular findings. However, immunoelectrophoresis revealed M protein of IgM-kappa type. In addition, the patient tested positive for RF. These findings strongly suggested the presence of CG.

Since clinical and pathological findings suggested cryoglobulinemia, we repeatedly tested the samples for CG but were unable to detect it. Thus, we suspected the presence of sCG and decided to store the samples for 2 weeks, which is longer than the regular storage time frame, and to examine the supernatant fluid from the serum samples for protein fractionation. In the turbid serum that had been cooled from 37°C to 4°C, a spike was observed in the γ fraction (arrow). After sedimentation, the spike disappeared, and the presence of CG was confirmed (Fig. 6).

Based on these findings, we proposed treatment using steroid therapy, which is often used in the treatment of CG. However, our patient refused steroid therapy. Thus, the patient was treated with an ARB. Following treatment, the patient experienced an uneventful course for 4 years, and her creatinine level is maintained at approximately 1 mg/dL.

Discussion

CGs are a type of cryoprecipitates that precipitate at 37°C or lower and redissolve on warming. Many patients with cryoglobulinemia are asymptomatic; however, symptomatic cases are referred to as CG syndrome (or CG disease). In particular, CGs form immune

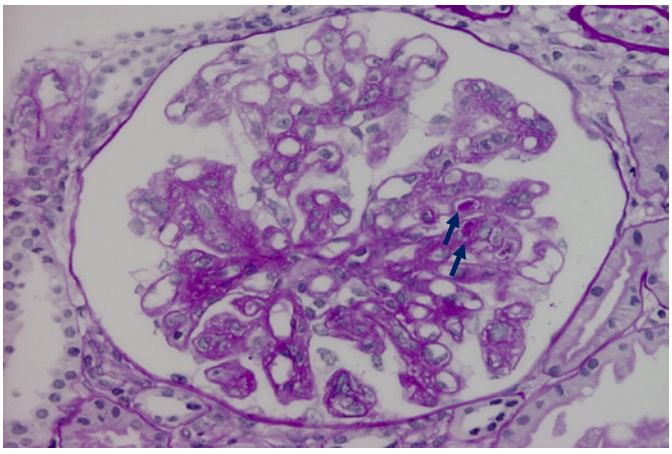

Fig. 1. Renal biopsy findings (PAS staining, medium magnification ×100): prominent lobularization, intraductal cell proliferation, mesangial cell proliferation, and diffuse stromal hyperplasia are observed. Hyaline thrombi (arrow) are also scattered.

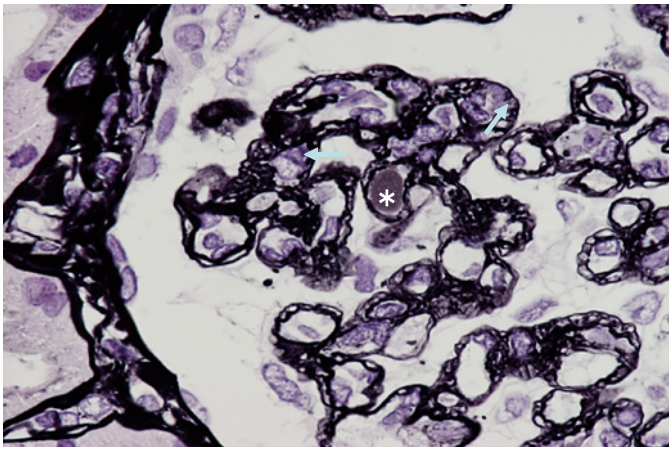

Fig. 2. Renal biopsy findings (PAM staining, strong magnification ×400): diffuse doubling, subendothelial deposits (arrows), and hyaline thrombi (asterisk) are observed.

complexes, promote the activation of complements, and may induce vascular endothelial cell injury through production of RF. Such injury is referred to as CG vasculitis.

The kidney is one of the target organs of CGs. Brouet et al. [3] have reported that type I cryoglobulinemia is not associated with characteristic renal lesions, but that renal lesions are observed in 31% of patients with type II and 12% of patients with type III. Although the prevalence of renal lesions slightly varies with subsequent reports, renal lesions have been observed in 8–58% of cases. The prevalence of renal lesions is higher than that initially expected. Approximately, 15% of renal lesions reportedly progresses to chronic renal failure [4, 5]. Progression to renal failure has been reported to affect fatal prognosis. Therefore, the diagnosis and treatment of cryoglobulinemia are extremely important. Two possible mechanisms have been suggested for the development of CG nephropathy: an occlusive disorder due to hyperviscosity syndrome or thrombosis and a disorder due to inflammatory reactions with immune complexes and vasculitis.

Detecting CG in blood or tissues is essential for diagnosing cryoglobulinemia and CG nephropathy. Slightly high false-negative rate is a problem. First, when CG levels are low, the

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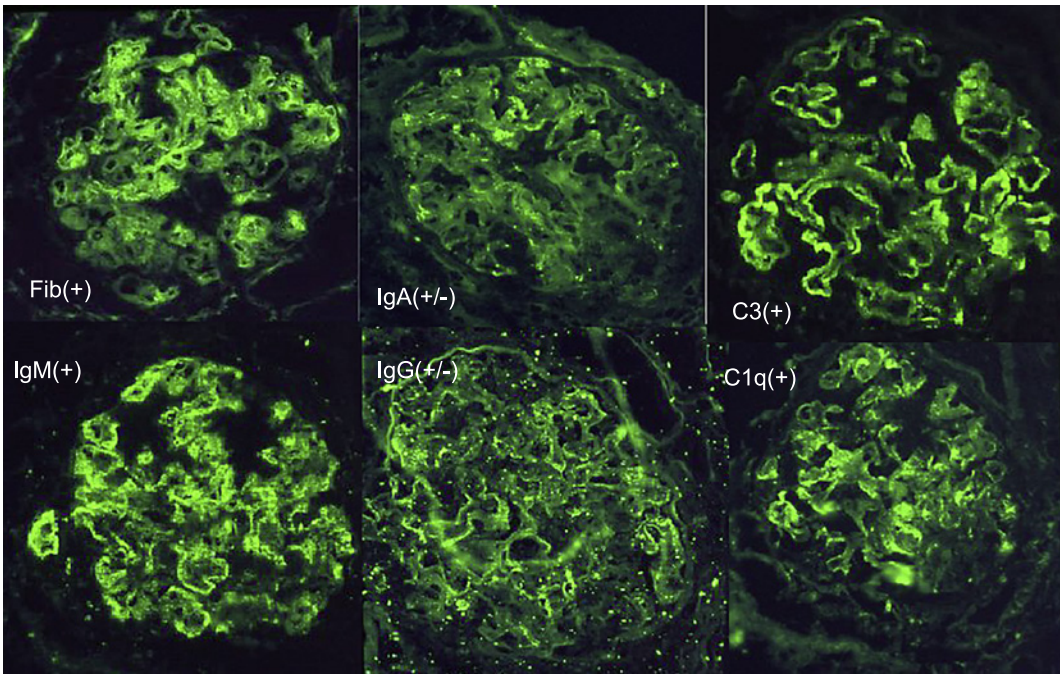

Fig. 3. Immunofluorescence findings: diffuse, loop-predominant, full-house EDD deposition is observed.

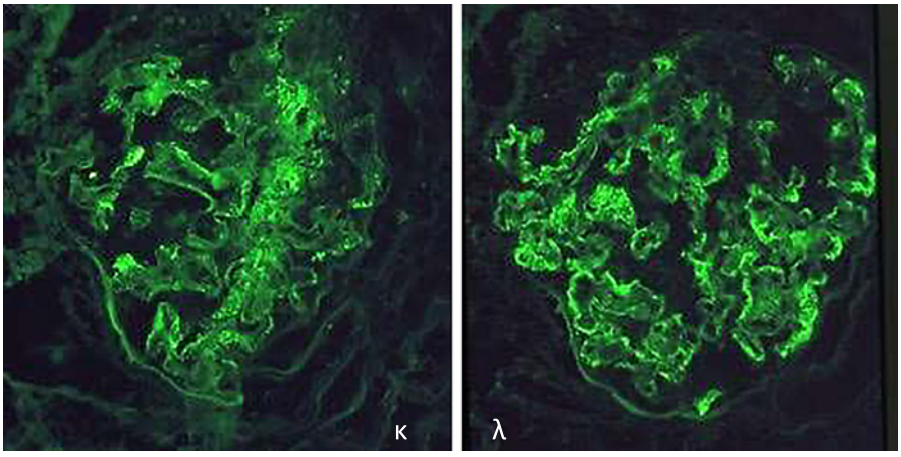

Fig. 4. Immunofluorescence findings: No κ/λ light chain deposition is observed.

detection limit becomes a problem. A study that carefully measured CG levels has shown the frequency of low CG levels to be much higher than previously reported frequency [6]. However, the impacts of low CG levels on the pathology are unknown. Another reason for the high false-negative rate is that the methods for collecting and storing samples are inadequate to measure CG levels. If samples are left at room temperature, CG will precipitate. The precipitates are separated by centrifugation, and a false-negative result is obtained. Furthermore, it should be noted that there are some cases in which it takes much time for CG to precipitate (i.e., sCG).

According to the general method for detecting CG, samples are placed still in a thermostat at 37°C and then centrifuged at approximately 3,000 revolutions. The resulting samples are divided into 2 portions. One is placed still at 4°C, and the other at room temperature (control)

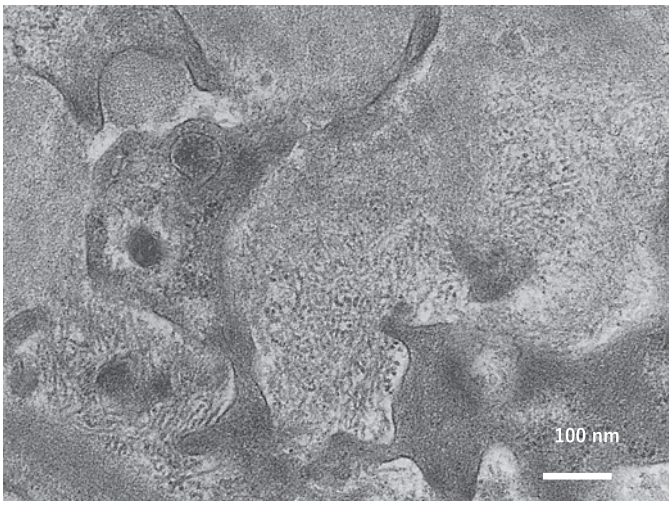

Fig. 5. Electron microscopy of a renal biopsy. Specimen viewed under ×20,000 magnification: Numerous EDD deposits are observed in the mesangial matrix and subendothelium (arrows). Inside is a fine fiber-like tubular structure, measuring 20–30 nm in diameter. These structures are considered to be CG based on their thickness and shape.

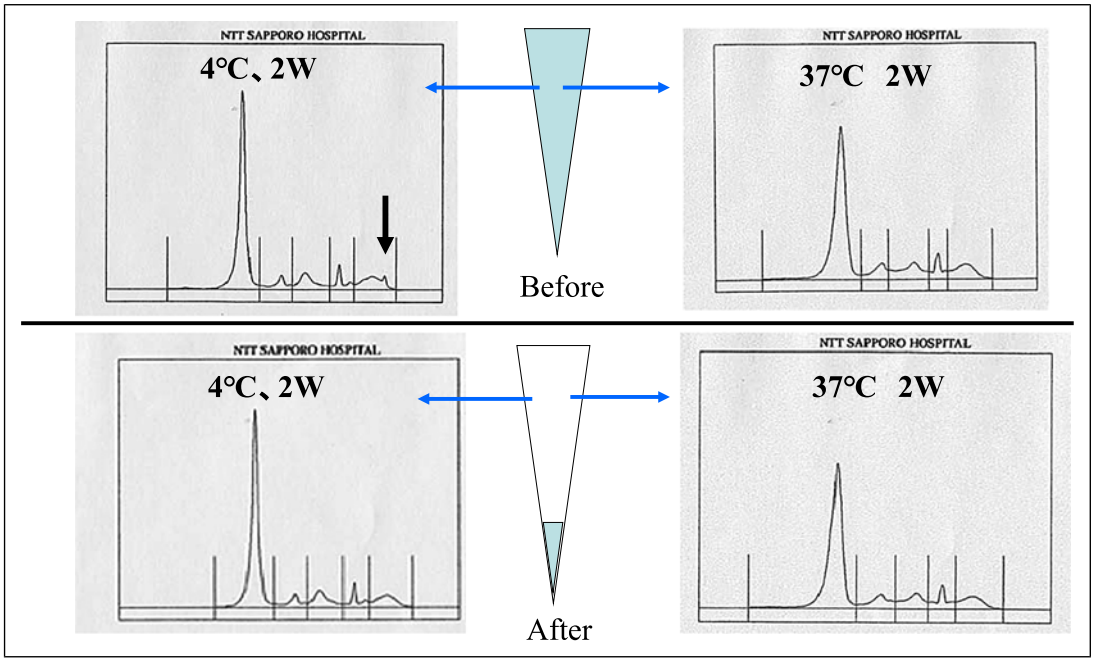

Fig. 6. The supernatant fluid from the serum samples for protein fractionation. Upper: turbid serum (before sedimentation) lower: after sedimentation: spikes were observed in the γ fraction, as indicated by the arrow, in the turbid serum cooled from 37°C to 4°C. These spikes disappeared after centrifugation, indicating the presence of CG.

for 1–3 days. When precipitates are produced in the samples that have been cooled at 4°C, the precipitates are warmed to 37°C. If they dissolve, they will be identified as CG. There are no internationally standardized methods for detecting CG, and at present, the methods vary among countries and laboratories. Thus, storage time frame at 4°C is not yet established.

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CG that precipitates only in samples placed still at 4°C for an extended period of time is referred to as sCG. In our present case, few precipitates were produced when the samples were placed still for approximately 3 days according to the regular storage time frame. However, CG precipitated when the samples were placed still for 2 weeks. However, the mechanism by which CG precipitates slowly over a long period of time is not clear.

Davie et al. first reported sCG in 1968. It is defined as CG that requires longer exposure to cold than the common types. There are also reports describing that no CG precipitation occurs until exposure to cold for 1 month or longer. Many aspects of sCG remain unknown. Some reports have indicated that such CGs showing delayed precipitation may be associated with hepatitis C and fibrillary glomerulonephritis [7, 8]; however, this is only speculation that is difficult to prove. Only a few reports are available regarding sCG, but its clinical picture is completely unknown. This case is progressing without severe vasculitis without immunosuppressive therapy such as steroids. Whether this mild course is clinically characteristic of sCG cannot be determined. Further accumulation of such cases is needed.

Based on our experience in this case, we believe that detection of sCG requires at least 2 weeks of observation. When there is a discrepancy between a pathological diagnosis and clinical symptoms as observed in our case, sCG should be taken into consideration, and careful examination is needed to detect it.

Conclusion

We experienced a case of essential mixed cryoglobulinemia due to sCG. Clinicians should be aware that some CGs require time to settle.

Statement of Ethics

The patient had provided written informed consent to publish her case (including publication of images). Ethical approval is not required for this study in accordance with local or national guidelines.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Seiji Hashimoto writes the manuscript, literature search, and submits the article. Yuichiro Fukasawa and Akira Suzuki specialize in pathology and performed pathological analysis. Nobuhiko Okamoto, Tomochika Maoka, and Rie Yamamoto are actually treating this patient. Sinichi Araki and Takao Koike provided supervision and mentorship. All authors substantially

contributed to the manuscript's conception and design. All authors contributed to drafting the manuscript or revisiting it critically for important intellectual content. All authors read and approved the final version of the manuscript. All authors agreed to be accountable for all aspects of the work.

Data Availability Statement

The data that support the findings of this case report are included in this article. Further inquiries can be directed to the corresponding author.

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