Cryoglobulinemic Vasculitis in Disguise: Cryofibrinogenemia as Variant of Monoclonal Gammopathy of Renal Significance

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Monoclonal gammopathy with cryoactivity (ie, cryoglobulins) that causes glomerulonephritis is considered within the spectrum of monoclonal gammopathy of renal significance. Cryofibrinogenemia (cryoactivity of coagulation factors) is very rarely associated with glomerulonephritis. We present a 39-year-old woman with a relapsing nephrotic syndrome. Laboratory investigation detected cryofibrinogen; the precipitate consisted of fibrinogen and a monoclonal immunoglobulin (M-protein; IgG-λ), and the latter was also detected in serum (4 g/L). Initial conventional immunosuppressive therapy resulted in temporary renal remission. In view of the M-protein, subsequent therapy consisted of bortezomib/dexamethasone and high-dose melphalan followed by autologous hematopoietic stem cell transplantation, and resulted in a very good partial hematological response and temporary renal remission. However, after hematological and renal relapse, we performed unique experiments to clarify the role of the M-protein. Mixing patient serum with donor plasma resulted in cryoactivity, composed of M-protein + fibrinogen. Patient plasma deprived of M-protein did not have cryoactivity. Therefore, cryoactivity was dependent on the M-protein. We started lenalidomide, which resulted in very good partial hematological and renal remission. Thus, cryofibrinogenemia can be the consequence of an M-protein, which we suggest should be defined as monoclonal gammopathy of renal significance.

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Introduction

Cryoproteins are proteins that reversibly precipitate at temperatures below 37°C, and can cause vasculitis-like and (micro)thrombotic disease.[1-3] The 2 main types of cryoproteins are cryoglobulins and cryofibrinogen. Cryoglobulinemia is a rare, although well-known, cause of glomerulonephritis.[4] Cryofibrinogenemia is even more rare than cryoglobulinemia, and is a relatively unknown cause of glomerular disease.[5] Cryofibrinogenemia is defined by cryoactivity after cooling of plasma, but not of serum (obtained after clotting of blood), which is due to dependency on coagulation factors such as fibrinogen, fibronectin, or factor VII.[1,6] As cryoactivity generally is determined only in serum, cryofibrinogenemia may often be unrecognized.[7]

Cryofibrinogenemia can be essential (30%-60% of cases), but also secondary to other conditions, such as hematological or solid malignancies and infectious or autoinflammatory diseases.[8-10]

Here we present a patient with relapsing nephrotic syndrome. Diagnostic work-up demonstrated cryofibrinogenemia and an M-protein in a very low concentration; kidney biopsy demonstrated membranoproliferative glomerulonephritis with weakly periodic acid–Schiff stain (PAS)-positive thrombi and immunoglobulin depositions. However, responses to both treatment for primary cryofibrinogenemia (ie, regular immunosuppressant therapy) and plasma cell clone–directed therapy were short-lasting. Therefore, we designed unique sophisticated experiments to investigate the relationship between cryofibrinogenemia and the M-protein. We proved that cryoactivity was dependent on the presence of the M-protein. The patient was diagnosed with M-protein-dependent cryofibrinogenemia, considered a novel type of monoclonal gammopathy of renal significance (MGRS), and treated accordingly.

Case Report

Fourteen years ago, a 39-year-old woman presented with joint pain and purpura. Her medical history revealed 2 pregnancies complicated by pre-eclampsia, but was further unremarkable. Autoimmune serology was negative (ANA, anti-dsDNA, ANCA, rheumatoid factor, cryoglobulins); C3, C4, and C1q were in the normal range; and serology for hepatitis B/C was negative. However, an M-protein (IgG-λ) was present in the serum (4.1 g/L). The patient had no myeloma- or lymphoma-associated symptoms, and bone marrow investigation did not show a monoclonal plasma cell population. Skin biopsy showed leukocytoclastic vasculitis; immunofluorescence staining was negative for IgG-λ. Consequently, the patient was diagnosed with cutaneous vasculitis of unknown cause and monoclonal gammopathy of unknown significance. Without treatment the vasculitis disappeared. During follow-up the patient sporadically had complaints of Raynaud's phenomenon, arthralgia, and muscle weakness.

Two years after initial presentation, the patient presented with nephrotic syndrome (proteinuria 10.6 g/24 h). Kidney function had remained normal, and autoimmune serology was again negative. Kidney biopsy showed membranoproliferative glomerulonephritis. The capillary loops contained numerous weakly PAS-positive

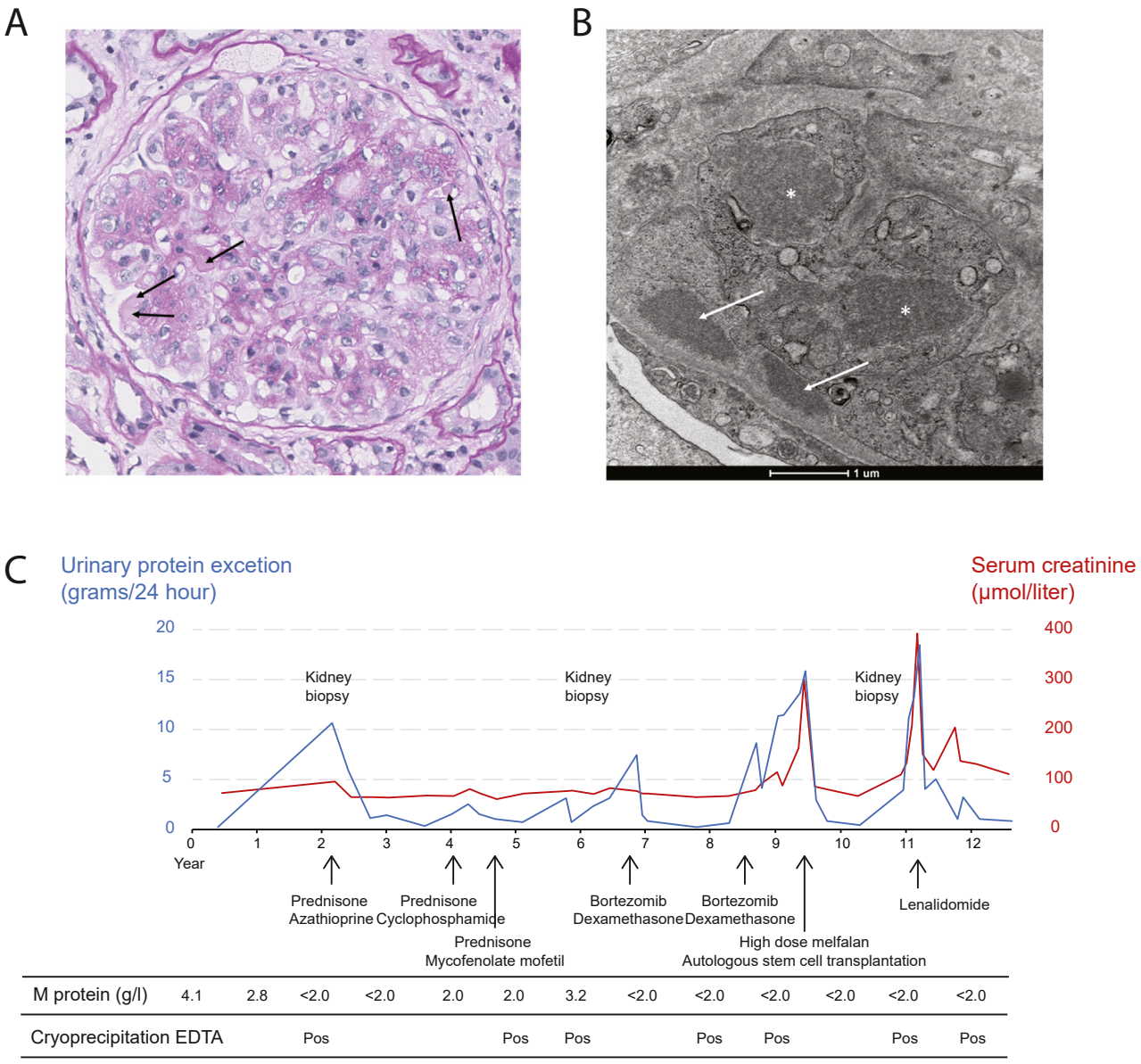

Figure 1. Kidney biopsy findings and timeline of renal parameters and treatment. (A) Light microscopy showed membranoproliferative glomerulonephritis with diffuse and global mesangial and endocapillary hypercellularity, double contours, and infiltration of leukocytes. The capillary loops contained numerous weakly periodic acid–Schiff-positive pseudothrombi (arrows). B. Electron microscopy showed multiple large electron dense deposits, mainly localized in the subendothelial area (arrows) and also in the capillary lumen (asterisks). (C) Timeline of proteinuria, renal function, m-protein, cryoproteins, and treatments. Pos, positive.

pseudothrombi (Fig 1A). Immunofluorescence demonstrated positive staining for IgG (1+), IgM (2+), C3c (1+), C1q (1+), kappa (1+), and lambda (2+), both in the pseudothrombi and along the glomerular basement membrane. Electron microscopy showed multiple large electron-dense deposits, localized in the subendothelial area, in the mesangium, and also in the capillary lumen (Fig 1B). Although the deposits lacked characteristic fibrillary or tactoid substructures, and the deposits were not strongly PAS positive, the kidney biopsy findings were most suggestive for cryoglobulinemic glomerulonephritis. A cryoglobulin assay was negative (Item S1). However, cooling of plasma resulted in cryoprecipitation, suggesting cryofibrinogenemia. Immunofixation electrophoresis of the precipitate identified 2 proteins: fibrinogen and the previously found IgG-λ M-protein. Of note, fibrin staining in the kidney biopsy was not performed. The patient was treated for cryofibrinogenemia with conventional immunosuppressive therapy (Fig 1C). Unfortunately, despite treatment escalation, proteinuria repeatedly relapsed. Therefore, 4 years later, the relationship between monoclonal gammopathy of unknown significance and

cryofibrinogenemia was re-evaluated. Bone marrow immunophenotyping now showed a small monoclonal plasma cell population (IgG-λ) was detected with immunophenotyping. We hypothesized that cryofibrinogenemia was in fact secondary to monoclonal plasma cell disease (MGRS). It should be noted that, as kidney biopsy was not suggestive for MGRS, the definite diagnosis remained inconclusive. We initiated bortezomib/dexamethasone, after which proteinuria improved. Three years later, nephrotic syndrome relapsed and the patient now had renal function impairment with an estimated glomerular filtration rate of 33 mL/min/1.73 m^2. We started high-dose melphalan, followed by autologous hematopoietic stem cell transplantation (Fig 1C). Thereafter, a very good partial response was achieved (serum M-protein <2.0 g/L), but cryofibrinogenemia–IgG-λ complex was still detectable in plasma. Importantly, kidney function improved and proteinuria abated.

Unfortunately, 2 years later, nephrotic syndrome relapsed with again significant deterioration of renal function (Fig 1C). We questioned whether the cryofibrinogenemia or the low M-protein level was the most relevant treatment target. Therefore, we performed additional unique analyses to provide definitive evidence.

We first confirmed that there was indeed no cryoactivity in serum (Fig 2A), but only in plasma (Fig 2B), with again fibrinogen and an IgG-λ M-protein. Blood examinations performed periodically demonstrated that this IgG-λ M-protein sometimes presented as a single band and sometimes as 2 monoclonal bands, representing both the monomeric and dimeric form of the IgG-λ M-protein, as illustrated in Fig 2B. The same monoclonal IgG-λ was detectable in full serum (Fig 2C).

Next, we did analyses to study the association between the M-protein and cryoactivity. We incubated patient serum (containing M-protein but not fibrinogen) with plasma from a healthy control (with no cryofibrinogenemia) at 4°C and observed a cryoprecipitate that was composed of fibrinogen and IgG-λ (Fig 2D). Here, we concluded that the M-protein IgG-λ had the capacity to induce cryofibrinogenemia in a healthy control. To exclude that other polyclonal antibodies or serum components could induce cryofibrinogenemia formation in our patient, we depleted all IgG-λ from the plasma sample using anti-λ magnetic beads. We observed no cryoformation in isolated plasma from the patient that was depleted of IgG-λ (Fig 2E). In addition to that, when the IgG-λ M-protein was eluted from the magnetic beads and was added to the plasma of a healthy control, the plasma of the healthy control formed a cryoprecipitate at 4°C composed of IgG-λ and fibrinogen (Fig 2F). From these findings we concluded that our patient secretes an IgG-λ M-protein that induces formation of cryofibrinogen.

Therefore, therapy directed at the plasma cell clone was started, consisting of lenalidomide monotherapy. Renal function and proteinuria improved, and remaining

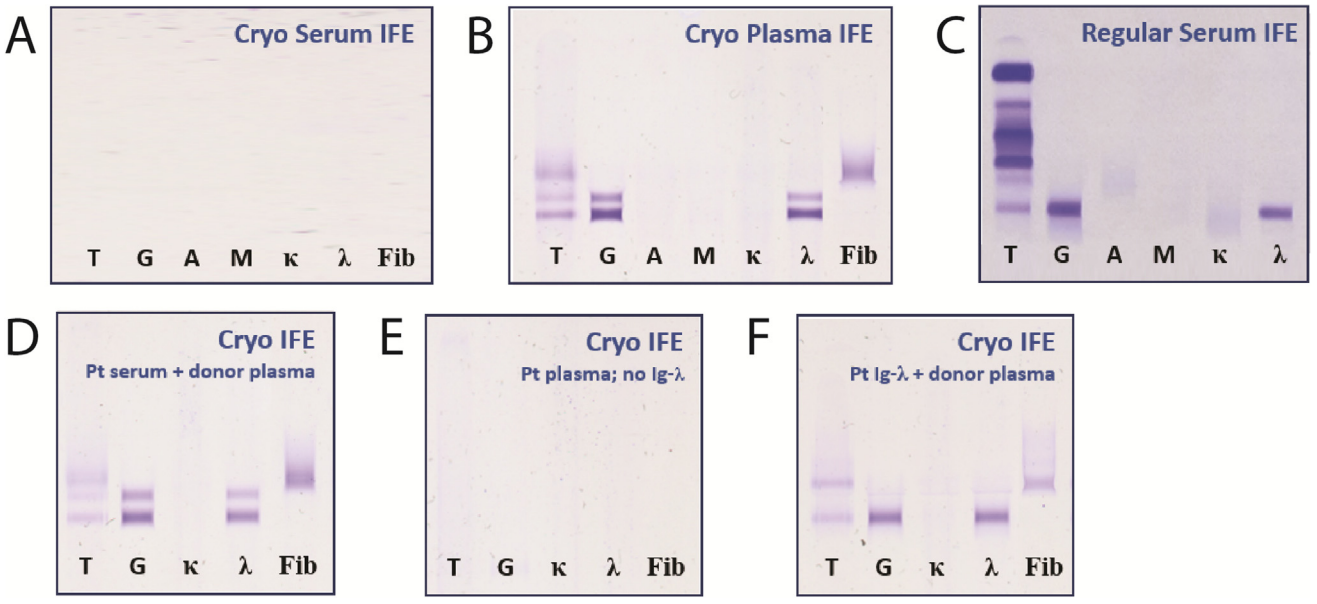

Figure 2. Cryoprotein analysis. (A) Negative cryoglobulin assay. (B) Positive cryofibrinogen assay; cryocrit is composed of monoclonal IgG-λ and fibrinogen. The monoclonal IgG-λ is sometimes present as single band and sometimes as 2 monoclonal bands, representing both the monomeric and dimeric form. (C) The IgG-λ M-protein is detectable using routine M-protein electrophoresis (<2 g/L). (D) Patient serum incubated with plasma from a normal individual at 4°C (1:1) induced a cryoprecipitate comparable to that formed in the patient plasma alone. (E) No cryoprecipitate was observed in patient plasma that was specifically deprived of λ-antibodies. (F) Spiking of the purified IgG-λ M-protein into plasma from a normal individual induced cryofibrinogen composed of the M-protein with fibrinogen.

symptoms of Raynaud's phenomenon, arthralgias, and muscle weakness became manageable. Serum IgG-λ M-protein remained stable in a low concentration (<2.0 g/L), with detectable cryofibrinogenemia–IgG-λ complex.

Discussion

We describe a case of M-protein-dependent cryofibrinogenemia associated with kidney disease. In literature, only few cases of M-protein-dependent cryofibrinogenemia are described, all with mainly cutaneous manifestations, and 1 with possible renal involvement.[11-14] We consider this a variant of monoclonal gammopathy of renal significance.

In M-protein-dependent cryofibrinogenemia a complex is formed consisting of fibrinogen and a monoclonal antibody with specificity for fibrinogen.[15] Interestingly, Nash et al[14] reported that in 5 of 10 studied patients with cryofibrinogenemia, the cryoactive substance included an M-protein, although none had a hematological malignancy, suggesting that monoclonal gammopathy of unknown significance might be a more important cause of cryofibrinogenemia than previously thought. Immunofixation of the isolated cryoprotein can be of added value, as illustrated in our case.

Of note, we cannot prove that the glomerular damage is caused by the cryofibrinogenemia. In case reports of cryofibrinogenemia, kidney biopsy demonstrated no immunoglobulin depositions, and there were unique morphologic ultrastructures on electron microscopy characterized by microtubular structures that have large central bores and double or triple layers.[5,16,17] In contrast, we did find immunoglobulin depositions and did not find such typical ultrastructures. Although initially a diagnosis of cryoglobulin-induced glomerulonephritis was suggested, it has to be noted that pseudothrombi due to cryoglobulins are typically very strongly PAS positive and not weakly positive. Thus in retrospect, the pseudothrombi observed in this case might represent the complex of fibrinogen and monoclonal IgG-λ. Taken together, it might be more likely that the glomerular deposition of the M-protein, in a complex with fibrinogen, is responsible for the kidney injury. Indeed, it was notable that kidney involvement first occurred 2 years after the initial presentation in 2009, when there was only cutaneous involvement of cryoactivity. In 2009, immunoglobulin staining of the skin biopsy specimen was negative. At that moment (2009), bone marrow biopsy did not show a monoclonal B-cell or plasma cell population. In contrast, in 2015 a very small monoclonal plasma cell population (0.3%) was detected. Possibly the underlying plasma cell disease evolved during the course of the disease, leading to differences in quality or quantity of the detected M-protein and, consequently, the composition of the cryoprecipitate.

It is still unclear why renal relapses were not heralded by increase of M-protein quantity, and why cryofibrinogenemia remained detectable during the entire follow-up of the patient (Fig 1C). We believe that the relapse may be dependent on the immune complex formation and subsequent complement activation. Interestingly, the complaints of the patient (Raynaud) paralleled the kidney relapses better than changes in serum M-protein concentration, compatible with the presence of larger immune complexes/cryoprecipitate. This would also explain positive polyclonal IgM staining in the kidney biopsy—we hypothesize that IgM deposition in this patient occurs after/in response to deposition of IgG-λ M-protein–dependent cryofibrinogen—and initial treatment effect of immunosuppressive therapy. The longer duration of renal remission after high-dose melphalan in turn suggested that a low level of the M-protein was tolerated in the pathophysiological process of cryofibrinogenemia-induced kidney damage. The trigger or tipping point after which immune complex formation starts and renal damage occurs is unknown.

In conclusion, we describe MGRS caused by M-protein-dependent cryofibrinogenemia. Clinical awareness for variants of MGRS is of utmost importance, as this has important consequences for treatment, prognosis, and follow-up. In MGRS, close collaboration between the nephrologist, hematologist, immunologist, and pathologist is vital both in establishing the diagnosis and treatment and in follow-up of the patient.

Supplementary Material

Supplementary File (PDF)

Item S1: Analysis of cryoproteins

Article Information

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