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Rituximab-Induced Remission in Epstein–Barr Virus–Associated Glomerulonephritis

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INTRODUCTION

Epstein–Barr virus (EBV), also known as HHV-4, is a ubiquitous oncogenic lymphotropic gamma herpes virus that infects the majority of adults. It evades the immune system, staying dormant in B-lymphocytes. Reactivation occurs in situations of stress and immunosuppression. Epstein–Barr virus can cause infectious mononucleosis, several types of lymphoma, and nasopharyngeal carcinoma. It has also been associated with autoimmune diseases such as systemic lupus erythematosus and multiple sclerosis.[1] Rare case reports describe the occurrence of acute interstitial nephritis,[2] acute tubular necrosis,[3] membranoproliferative glomerulonephritis (MPGN),[4] minimal change disease,[5] and membranous nephropathy[6] in the setting of EBV infection. There remains, however, a scarcity in the literature about the association between EBV and glomerulonephritis (GN). Reactivation of EBV has been implicated in the pathogenesis of monoclonal gammopathy, particularly when T cells are depleted with anti-thymocyte globulin or alemtuzumab, as in stem-cell transplantation.[7] Monoclonal proteins, through their physicochemical properties or via immunological mechanisms, can cause a variety of renal diseases collectively labeled as monoclonal gammopathies of renal significance (MGRS).[8] Although some antiviral drugs have activity against EBV, none has been approved so far for its treatment.[9]

Immune-complex mediated MPGN (IC-MPGN) is a rare form of GN characterized by the presence of subendothelial nephritogenic immune complexes in the glomerular capillary wall, mesangial interposition, and activation of the classical complement pathway. It can be idiopathic or more commonly secondary to infections, autoimmune diseases, or paraproteinemia.[S1] The coexistence of all 3 factors is distinctly unusual. Treatment of MPGN depends on the cause. Infections such as hepatitis C or B are treated with antivirals. Immunosuppression in such cases can be deleterious. Neoplasms such as chronic lymphocytic leukemia are treated with chemotherapy or biotherapeutics, whereas autoimmune diseases are treated with immunosuppressive drugs. The proper treatment of monoclonal gammopathy of renal significance remains uncertain.[S2]

CASE PRESENTATION

A 51-year-old woman presented with anasarca and dyspnea following a flu-like illness. She was found to have severe new-onset hypertension, pulmonary edema, and bilateral pleural effusions. She had no B-symptoms, lymphadenopathy, or splenomegaly. Urinalysis revealed an active sediment with 3+ protein and 3+ blood. Her 24-hour urine protein was 3.63 g and serum albumin was 35 g/l. Serum creatinine was within normal range at 85 μmol/l. C4 was normal, but C3 was low. Connective tissue serologies were negative. Infectious serologies were positive for EBV early and nuclear antigens with a negative IgM. She was treated with i.v. furosemide and required 4 additional antihypertensive drugs, including a renin–angiotensin system (RAS) inhibitor. Renal biopsy revealed marked endocapillary hypercellularity, neutrophils, and

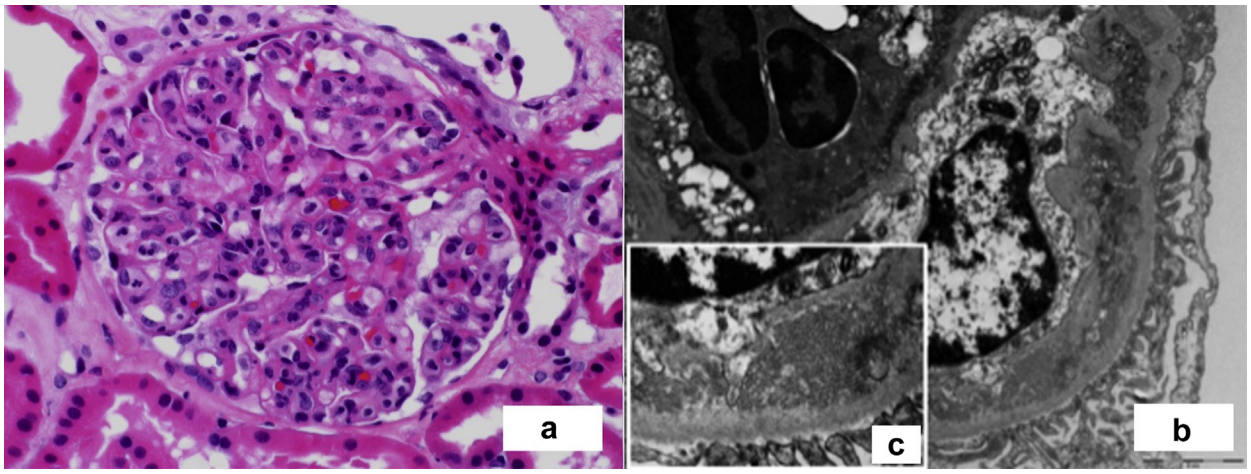

Figure 1. (a) A glomerulus showing endocapillary hypercellularity with neutrophil infiltration (hematoxylin and eosin stain). (b) A glomerulus showing subendothelial electron dense deposits (electron microscopy; low magnification). (c) Higher magnification of Figure 1b demonstrating organized substructure of these deposits with annular-tubular arrays appearance.

monocytes infiltration, and focal karyorrhexis but no crescents (Figure 1a). Immunofluorescence study showed granular glomerular capillary wall staining with antisera specific for IgG (1+), IgM (2+), C3 (3+), C1q (1+), and kappa (3+) and lambda (1+) light chains. Staining for IgA and fibrinogen was negative. On electron microscopy, the glomeruli showed multiple subendothelial electron-dense deposits with focal mesangial interposition, basement membrane duplication, and occasional mesangial deposits. Some of the deposits demonstrated organized substructure composed of annular-tubular arrays suggestive of cryoglobulinemia (Figure 1b and 1c). Cryoglobulins were negative on 2 occasions. The EBV viral load was 18.948 copies/ml. Table 1 lists laboratory findings upon admission.

The initial diagnostic impression was acute GN secondary to EBV infection, and thus no immunosuppressive therapy was prescribed. Over the following 3 months, proteinuria decreased to a protein/creatinine ratio (P/Cr) of 1.67 g/g, serum albumin and complement levels increased, and renal function remained stable. Although serum and urine protein electrophoresis were negative, free kappa light chains were present on urine immunofixation. Serum level of free lambda chains was normal, whereas that of kappa chains was increased with κ:λ ratio of 5.84 (N 0.26−1.65) consistent with the presence of paraproteins. Bone marrow biopsy findings were normal, with 2% plasma cells. Light chain restriction could not be assessed.

Liver enzymes increased in a mixed pattern, and ultrasound showed cholelithiasis, whereas magnetic resonance cholangiopancreatography (MRCP) was negative for choledocholithiasis. This was followed by a nephrotic relapse accompanied by a marked decrease in C4 levels and an increase of EB viral load to 34,600 copies/ml. Oral prednisone 60 mg daily was initiated when the P/Cr showed a marked increase to 13.5 g/g but was soon reduced to 40 mg daily because of poor tolerance. Despite a transient decrease in P/Cr to 3.94 g/g over the following 2 months, EB viral load increased further to 94,600 copies/ml. The patient was administered a dose of rituximab (700 mg), and prednisone was further tapered. Proteinuria continued to decrease, reaching a P/Cr of 1.64 g/g months later but then increased again to P/Cr of 11.36 g/g with a decline in eGFR to 34 ml/min (Modification of Diet in Renal Disease [MDRD] equation). Additional doses of rituximab (1 g each) were given, allowing for a brief CD-19 cell recovery in between. Tacrolimus was added, maintaining a level of 3.5 to 5 mg/l over the following year. By 6 months after the first rituximab dose, the EB viremia had completely resolved. Proteinuria continued to decrease and estimated glomerular filtration rate (eGFR) to increase, both returning to normal in less than 1 year. Urinalysis, complement levels, free kappa light chain, and κ/λ ratio all normalized. Antihypertensive drug requirements decreased to 1 drug. Three-and-a-half years after the last dose of rituximab, the patient remains in complete clinical, biochemical, hematological remission, with no detectable viremia. Subsequent *in situ* hybridization on the kidney tissue was negative for EBV. Figure 2 tracks changes in laboratory results, while showing therapeutic interventions from the time of diagnosis.

DISCUSSION

Glomerulonephritis is an inflammatory condition in which tissue damage is mediated by antibodies, immune complexes, and/or complement activation. It can be precipitated by infection or chemical

Table 1. Patient's admission laboratory results

CBC differential		
WBC	5.74	10^9/l
RBC	L 3.87	10^{12}/l
HGB	111	g/l
HCT	0.32	l/l
MCV	82.7	fL
PLT	210	10^9/l
Peripheral blood morphology	Few reactive lymphocytes and mild thrombocytopenia	
Urine chemistry		
Creatinine, random urine	11.68	mmol/l
Protein, random urine	4.29	g/l
PCR, random urine	3.23	g/g
Urinalysis		
Protein	3+	
pH	6.5	
Blood	3+	
WBC	6-10/hpf	
RBC	31-40/hpf	
Infectious serology		
Hepatitis Bs antigen	Nonreactive	
Hepatitis C virus antibody	Nonreactive	
Streptozyme	Negative	
CMV IgM antibody	Nonreactive	
Parvo virus B19, IgM antibody	Nonreactive	
Parvo virus B19, IgG antibody	Reactive	
EBV IgM	<10 (n = <20)	U/ml
EBV early antigen	135 (n = <9)	U/ml
EBV nuclear antigen	54 (n = <5)	U/ml
Autoimmune serology		
C-ANCA (PR3)	<3.1	U/mL
P-ANCA (MPO)	<3.1	U/mL
ANCA IF	Negative	
ANA	Negative	
Anti-GBM antibody	Negative	Units
Cryoglobulin	Absent × 2	
Rheumatoid factor	Not done	
Protein electrophoresis, serum	No monoclonal spike	
Protein electrophoresis, urine	No monoclonal spike	
Urine immunofixation	Free kappa light chains	

ANA, antinuclear antibody; ANCA, antineutrophil cytoplasmic antibody; EBV, Epstein–Barr virus; CBC, complete blood count; CMV, cytomegalovirus; GBM, glomerular basement membrane; HCT, hematocrit; HGB, hemoglobin; IF, immunofluorescence; MCV, mean corpuscular volume; MPO, myeloperoxidase; PCR, polymerase chain reaction; PLT, platelet; RBC, red blood cells; WBC, white blood cell.

exposure; however, the trigger is often unclear. Among different infectious agents, hepatitis B, hepatitis C, and human immunodeficiency viruses are commonly associated with glomerular diseases.[S2] Although many humans are infected with EBV, it is rarely implicated as a cause of GN. However, EBV has been associated with cases of GN, interstitial nephritis, and monoclonal gammopathy.[4] In some of those cases, the presence of the virus was demonstrated in the kidney. EBV has a special ability to transform B-lymphocytes, reliably and efficiently.[S3] This can explain its association with lymphomas as well as with autoimmune diseases. Paraproteins are markers for the clonal proliferation of B-lymphocytes and plasma cells and can mediate a number of renal diseases when they deposit in kidney tissue.[8] A monoclonal protein can also be a target antigen for antibodies, leading to complement activation and vasculitis, as in the case of type II cryoglobulinemia. Alternatively, it can directly activate complement, as in some cases of type I cryoglobulinemia and proliferative GN with monoclonal immunoglobulin deposition.[S4]

Our patient is unique in that the acute GN was accompanied by a progressive EB viremia and kappa light chain proteinuria. Immunofluorescence suggested the presence of immune complexes of a monoclonal IgM kappa and a polyclonal IgG in a manner akin to rheumatoid factor and type II cryoglobulinemia. Although serum cryoglobulins were negative on 2 occasions, the presence of the characteristic ultrastructure on EM was highly suggestive. The negative *in situ* hybridization argues against a direct cytopathic effect of EBV on the kidney. It is more plausible that a clonal transformation of a B-lymphocyte resulted in the production of a small amount of IgM kappa paraprotein that acted as a neoantigen, inducing a humoral immune response as in the paraneoplastic syndromes.

Epstein–Barr virus infection in humans is mostly in a latent phase as a result of the expression of specific viral proteins. Reactivation occurs as the virus enters a phase of lytic replication and is important in the pathogenesis of some EBV-related diseases. Glucocorticoids have been shown to induce lytic replication by a dose-dependent upregulation of the expression of the immediate early gene BZLF1, which produces the protein Zebra.[1,S5] The source of glucocorticoids can be endogenous, as in stress situations, or exogenous when taken for a therapeutic indication, as is often the case in idiopathic or autoimmune-associated MPGN. On the other hand, rituximab, by depleting B cells, can reduce the risk of EBV-associated diseases, as has been reported in immunosuppressed organ transplant recipients.[S6] Rituximab has also been proposed to treat monoclonal gammopathies when the source of the culprit clone is a lymphocyte, as in the case of macroglobulinemia of Waldenstrom, as opposed to that of a plasma cell as in multiple myeloma.[S2]

Our patient's kidney disease was preceded by a flu-like illness consistent with a viral prodrome. Epstein–Barr virus viremia was documented early in her disease course and increased with her first nephrotic relapse. The patient's age and the absence of IgM EBV antibodies support a reactivation rather than a primary EBV infection. Following a brief partial nephrotic remission with prednisone, she had a severe

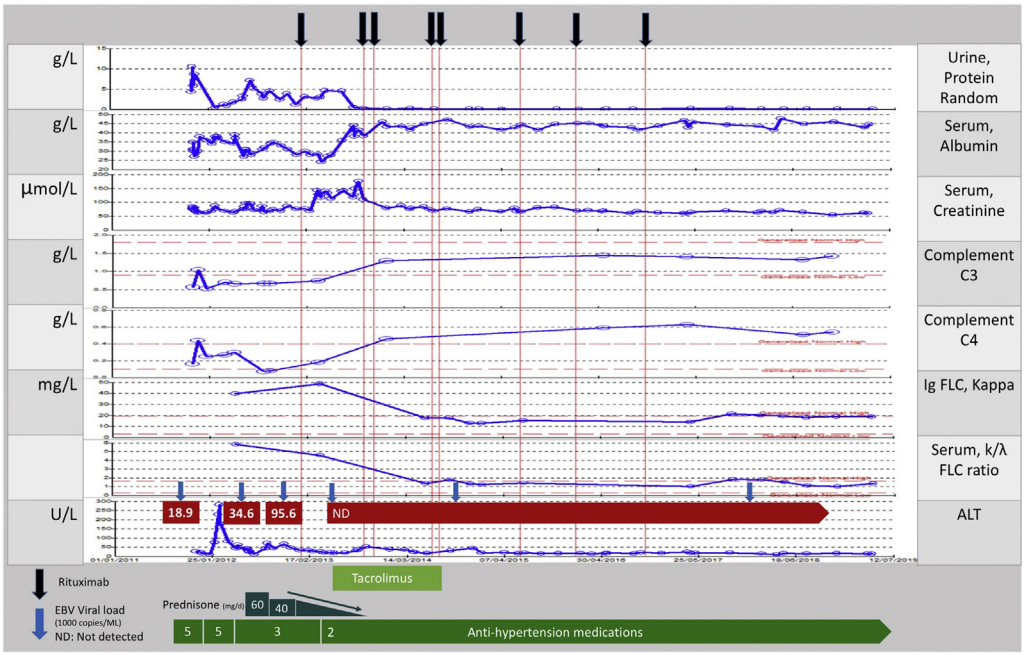

Figure 2. Relevant laboratory results and therapeutic interventions from the time of presentation until the last follow-up.

nephrotic relapse, and the EB viral load increased to 94,600 copies/ml. Following the first dose of rituximab, EB viremia completely resolved and the proteinuria improved. With subsequent doses, the patient achieved a complete remission in all disease manifestations including urinary protein excretion, hematuria, renal impairment, hypocomplementemia and paraproteinemia. This persisted for more than 3 years following the last dose of rituximab.

Our patient presented in 2011, when the evidence for rituximab use in kidney diseases was limited. The initial immunosuppression used consisted of tapered glucocorticoids, and tacrolimus was added subsequently.[S7] In the absence of a standard rituximab regimen for MPGN, we dosed it every 6 to 9 months while monitoring for B-cell recovery and immunoglobulin levels. The treatment continued until the complete resolution of disease activity with several doses afterward to reduce the possibility of a clonal disorder relapse.

In conclusion, we present a case of lytic reactivation of EBV infection associated with a probable IgM kappa monoclonal gammopathy in the form of MPGN-Ig with type II cryoglobulinemia-like features. The kidney disease failed to respond to glucocorticoid therapy, which enhanced viral replication and exacerbated the glomerulopathy. Treatment with a multi-dose rituximab regimen achieved a complete durable clinical, biochemical, and hematological remission (Table 2). The co-occurrence of EBV infection, monoclonal gammopathy, and the renal pathological findings as well as the parallel responses to therapeutic interventions suggest that our case represent an MGRS. As in type II cryoglobulinemic glomerulonephritic MGRS, the mechanism of renal injury is indirect, and a circulating IgM kappa monoclonal protein cannot be found in about half of the cases.[S8,S9] The mounting evidence of the relationship between EBV and paraproteinemia provides additional support.

Table 2. Teaching points

1. Patients with GN including MPGN should be evaluated for underlying infections, autoimmune diseases, and paraproteinemia. If no secondary cause can be identified, it is labeled as idiopathic.
2. Treatment of secondary GN should be directed at the underlying etiology.
3. EBV can cause GN by several mechanisms, including the transformation of B-lymphocytes, resulting in the development of a monoclonal gammopathy and the subsequent formation of nephritogenic immune complexes.
4. The use of glucocorticoids and other immunosuppressive agents in infection-associated GN can result in the exacerbation of the infection and worsening of the kidney disease.
5. Rituximab, by depleting B-lymphocytes, has a unique role in the treatment of EBV-associated pathology by clearing both the viremia and the pathogenic paraprotein-producing clone.

EBV, Epstein–Barr virus; GN, glomerulonephritis; MPGN, membrano-proliferative glomerulonephritis.

DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary References.

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