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. Rare case reports describe the occurrence of acute interstitial nephritis, acute tubular necrosis, membranoproliferative glomerulonephritis (MPGN), minimal change disease, and membranous nephropathy 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. 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). Although some antiviral drugs have activity against EBV, none has been approved so far for its treatment.

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. 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.

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
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 $\kappa:\lambda$ 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 cholangiopancreatoscopy (MRCP) was negative for cholecoddolithiasis. 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 $\kappa:\lambda$ 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              | 3.87 10^9/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 |
|                  | PÇR, random urine 3.23 g/l |
|                  | Proteinuria Protein 3+ |
|                  | pH 6.5 |
|                  | Blood 3+ |
|                  | WBC 6-10/hpf |
|                  | RBC 31-40/hpf |
| Infectious serology | Hepatitis B antigen Nonreactive |
|                  | Hepatitis C virus antibody Nonreactive |
|                  | Streptolzyme 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 |
|                  | Cryoglobulin Absent |
|                  | 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.

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. 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. 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. The source of glucocorticoids can be autoantibodies, reliably and efficiently.

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 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. 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. 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. This can explain its association with lymphomas as well as with autoimmune diseases. Paraproteins are
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. 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. The mounting evidence of the relationship between EBV and paraproteinemia provides additional support.

**DISCLOSURE**

All the authors declared no competing interests.

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**SUPPLEMENTARY MATERIAL**

Supplementary File (PDF)

**Supplementary References**
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