Use of Rituximab in Conjunction With Immunosuppressive Chemotherapy as a Novel Therapy for Epstein Barr Virus-associated Hemophagocytic Lymphohistiocytosis

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Summary: Hemophagocytic lymphohistiocytosis is a rare, life-threatening complication of Epstein Barr virus (EBV) infection. Current treatments are directed at reducing virus-induced immune dysregulation. Addition of agents that eliminate EBV-infected B cells may improve therapeutic efficacy. On the basis of the observations that the anti–CD-20 monoclonal antibody rituximab reduces disease burden in individuals with EBV-associated lymphoproliferative disorders, we treated a patient with severe EBV-hemophagocytic lymphohistiocytosis using a combination of rituximab and chemotherapy. This patient demonstrated a rapid clinical response and an 18-fold reduction in EBV viral load within 24 hours of receiving rituximab. He remains free of disease 8 months after completing treatment.

Key Words: immunotherapy, Epstein Barr virus, rituximab, lymphohistiocytosis

(Hemophagocytic lymphohistiocytosis (HLH) is a rare disorder characterized by inappropriate activation of the immune system and release of proinflammatory cytokines.1 Patients with HLH typically present with symptoms of prolonged fever and hepatosplenomegaly, in association with abnormal laboratory values, such as pancytopenia, transaminitis, coagulopathy, hypertriglyceridemia, and hypercytokinemia. Without treatment, patients may rapidly deteriorate owing to the development of infection, bleeding complications, and/or multisystem organ failure.

HLH occurs either as a hereditary condition caused by germline mutations in one of several genes involved in cytolytic granule exocytosis, or as a nonhereditary disorder triggered by infection, malignancy, or autoimmune disease.2 Epstein Barr virus (EBV), the virus causing infectious mononucleosis, is the most common known infectious trigger of hereditary or nonhereditary HLH.2 In normal individuals, EBV infects and transforms B-lymphocytes, which are then destroyed by natural killer (NK) cells and cytotoxic CD8+ T cells.3 The pathogenesis of EBV-induced HLH (EBV-HLH) is poorly understood. One hypothesis is that EBV-infected B cells are not eliminated in a normal fashion by effector NK and CD8+ T cells. This leads to their accumulation and the subsequent activation of T lymphocytes and NK cells, which release proinflammatory and TH1-type cytokines including tumor necrosis factor-α and interferon-γ, resulting in the secondary activation of histiocytes and macrophages. In a subset of EBV-HLH patients, particularly individuals of Asian origin, EBV infects T or NK cells instead of B cells, which can lead to development of a polyclonal or monoclonal T or NK cell lymphoproliferative disorder.4

Although the prognosis of noninherited HLH is often less dismal than inherited HLH, EBV-HLH can have a high morbidity and mortality.5 The optimal treatment for EBV-HLH is unknown. Most reports describing successful outcomes for EBV-HLH patients have used immunosuppressive medications to inhibit overactive T and NK cell responses, in conjunction with chemotherapeutic agents to target dividing lymphocytes and mononuclear phagocytes.5 Intravenous immunoglobulin,6 corticosteroids, cyclosporine, and etoposide7 have been used as single agents, and in varying combinations, with survival rates ranging between 50% and 75%.5,8 Rituximab, a humanized anti-CD20 monoclonal antibody that targets mature B cells, is an effective treatment for a number of EBV-mediated conditions, including EBV-induced posttransplantation lymphoproliferative disorder.9 Recently, we used rituximab and corticosteroids to prevent the development of fulminant infectious mononucleosis, a condition resembling EBV-HLH, in 2 unrelated patients with X-linked lymphoproliferative disease (XLP).10 On the basis of these findings, we hypothesized that the addition of rituximab to more conventional therapies would provide an effective treatment for EBV-HLH. Therefore, we used rituximab in conjunction with immunochemotherapeutic agents to
control disease in a patient with severe EBV-HLH. Within 24 hours of initiating immunosuppression and receiving rituximab, our patient exhibited a marked improvement in symptoms and an 18-fold reduction in EBV viral load. He is currently disease free 8 months after completing therapy.

**CLINICAL COURSE**

Approval from our local institutional review board was obtained to examine this case retrospectively. A previously healthy 14-year-old boy presented with a 3-day history of fever, lymphadenopathy, and fatigue. He was diagnosed with lymphadenitis and treated with antibiotics without improvement. Two weeks later, he returned to his doctor because of persistent fevers, dysphagia and odynophagia. A monospot test was positive. After a 5-day course of prednisone, he experienced improvement in odynophagia but fevers continued. Two weeks later, he developed jaundice, abdominal pain, and nausea, and he was referred to the emergency room at our institution.

On physical examination, he was febrile to 38.6°C, ill appearing and had scleral icterus, petechiae, prominent bilateral cervical lymphadenopathy, hepatomegaly (5 cm below the right costal margin), and splenomegaly (4 cm below the left costal margin). Serum chemistry evaluation revealed a total bilirubin of 16.5 mg/dL, conjugated bilirubin 12.1 mg/dL, alanine aminotransferase 201 U/L, aspartate aminotransferase 247 U/L, ferritin 9070 ng/mL (normal 10 to 300 ng/mL), triglycerides of 281 mg/dL, and lactate dehydrogenase of 3675 U/L. A complete blood count revealed a white blood cell count of 5900/μL, hemoglobin 12.2 g/dL, hemophagocytic lymphohistiocytic syndrome, and an increased number of CD163+ macrophages with hemophagocytosis. Cerebrospinal fluid was normal. A complete blood count 12 hours after admission revealed a hemoglobin of 8.8 g/dL. Having met 6 of the 8 diagnostic criteria for HLH [fever, splenomegaly, cytophenias, hypertriglyceridemia (≥265 mg/dL), hyperferritinemia (≥500 ng/mL), and hemophagocytosis], treatment was started with daily oral cyclosporine (5 mg/kg) and dexamethasone (10 mg/m²). Genetic analysis revealed no mutations in *PRF1* and *MUNC13-4* or *SH2D1A*, genes defective in familial HLH and XLP, respectively.12 A NK cell functional assay revealed no intrinsic defect in NK cell cytotoxic activity. The soluble IL-2 receptor was not elevated. T and B cell functions were not assessed at the time of diagnosis.

Twenty-four hours later, the patient developed tachypnea, rales, and an escalating oxygen requirement, consistent with adult respiratory distress syndrome, and he was transferred to the intensive care unit. Laboratory evaluation revealed an increase in serum creatinine [1.2 mg/dL (baseline 0.4 mg/dL)], worsening coagulopathy (peak prothrombin time 17 s, peak partial thromboplastin time 45.9 s, and lowest fibrinogen 71 mg/dL) and a marked increase in ferritin (>10,000 ng/mL, the maximum level detectable by our laboratory). His EBV copy number also rose to 1.46 million copies/mL. Etoposide (150 mg/m²) was administered per HLH-2004 protocol11 and a single dose of intravenous rituximab (375 mg/m²) was added to his therapy.

Within 24 hours of receiving rituximab and a single dose of etoposide, and 48 hours after starting immunosuppression, the patient exhibited marked clinical improvement. He was weaned from oxygen and his fever curve began to decrease. His EBV copy number fell to 83,270 copies/mL. Laboratory values, including ferritin, fibrinogen, absolute neutrophil count, and bilirubin, all rapidly normalized (Fig. 1). He was discharged home on hospital day 11. On the basis of his rapid clinical response, on data suggesting that functional T cells are helpful in eradicating EBV in posttransplantation lymphoproliferative disorder,9 and because cyclosporine was not included in induction therapy in the previous Histioocyte Society HLH protocol, HLH-94, we elected to discontinue this medication after 2 weeks of therapy. The patient did, however, receive an 8-week course of induction chemotherapy with etoposide and dexamethasone, according to the HLH-2004 protocol, with the exception that the tenth dose of etoposide was held secondary to transient bone marrow suppression. A repeat bone marrow examination during this time showed a hypocellular marrow with trilineage hematopoiesis and no evidence of hemophagocytosis. The patient received 1 dose of granulocyte colony stimulating factor with excellent rebound of white blood cell count. As he was in remission and had no evidence for genetic mutations linked to familial HLH or XLP, he did not receive continuation therapy.

He is currently free of disease 8 months off treatment. EBV serology 6 months after therapy showed low, but detectable antiviral titers, including a viral capsid IgG of 1:40, early antigen IgG of 1:20, and Epstein-Barr nuclear antigen of 1:10. EBV copy number at the same time revealed 0 copies of EBV genome/mL.

**DISCUSSION**

The diagnosis and treatment of EBV-HLH are challenging. Because symptoms evolve rapidly, a low index of suspicion and rapid introduction of definitive therapy are required. Indeed, early treatment intervention substantially improves the outcome for children and young adults with EBV-HLH.2 Genetic testing for inherited conditions linked to hemophagocytosis, such as familial HLH and XLP should be considered, particularly in young (<1 y of age) or male patients with EBV-HLH.
Several reports have suggested that outcome is improved in patients with infection-triggered HLH if one targets the inciting pathogen. Further, it has been shown that EBV viral load correlates with disease activity in EBV-HLH, suggesting that persistence of elevated levels of pathogenic antigen drive the dysregulated inflammatory response that is typical of this disorder. The cytolytic activity of T and NK cells is critical for eliminating virus-infected target cells, including EBV-infected B cells. Patients with familial or secondary HLH commonly exhibit defective killing of target cells in vitro. Therefore, it is likely that they have difficulty in clearing virus-infected cells in vivo. On the basis of this possibility, we hypothesized that by reducing the load of circulating EBV-infected cells, we might be able to diminish the immunopathology associated with EBV-HLH.

Useful treatments to combat EBV include intravenous immunoglobulin, which would provide antibodies to neutralize the virus, or antiviral medications such as acyclovir or gancyclovir. Unfortunately, these agents have not been shown to be consistently active against EBV-HLH.

In both acute and latent EBV infection, the virus resides within the B cell compartment. Rituximab, a humanized monoclonal antibody that recognizes the B cell marker CD20, depletes CD20+ cells within 48 hours of therapy. Because of rituximab’s ability to quickly clear infected B cells, it has become an attractive option to decrease viral burden in a number of EBV-mediated diseases. In agreement with these data, our patient’s viral load dropped from >1,000,000 copies of EBV/mL blood to 83,000 copies/mL in only 24 hours after receiving a single dose of rituximab.

It is not sufficient, however, to target only the B cell population in EBV-HLH, especially in patients with signs of hypercytokinemia. Specifically, T cells, NK cells,
dendritic cells, and macrophages also need to be eliminated or suppressed. For this reason, effective treatment of HLH involves the use of traditional cytotoxic agents such as etoposide and immunosuppressants, including cyclosporine and prednisone. Although these immunochemotherapeutics are effective, they may be associated with side effects, many of which occur after prolonged courses of treatment. Cyclosporine can cause severe headache, hypertension, seizures, and renal impairment. Steroids, in addition to furthering immunosuppression, may cause hyperglycemia, fluid retention, hypertension, and weight gain. Etoposide is associated with myelosuppression and development of secondary leukemia. Rituximab was well tolerated by our patient and not associated with the development of obvious acute side effects. He did not develop any infusion reactions, including fever or rigors, which have been reported with rituximab. Although he developed a transient period of neutropenia, this side effect was more consistent with the cumulative effects of etoposide than rituximab, which has been linked to late onset neutropenia in certain patients. In support of this possibility, our patient’s bone marrow at the time of neutropenia showed hypocellularity suggestive of generalized myelosuppression and not depletion of myeloid cells or neutrophil maturation arrest, as described previously in rituximab-treated patients.

Repeated treatment with rituximab may lead to long-term suppression of immunoglobulin production; however, patients maintain the ability to produce specific antibodies. In our patient, anti-EBV titers were low, but detectable, 6 months after completing therapy. It will take time to determine whether this single dose of rituximab will impact upon the development of a longer-term anti-EBV immune response in this patient. Rituximab-treated patients have not been reported to have an increased risk of infection, with the exception of reactivation of certain viruses, including hepatitis B that can cause hepatic failure and JC virus that can lead to progressive multi-virus, including hepatitis B that can cause hepatic impairment. Steroids, in addition to furthering immunosuppression, may cause hyperglycemia, fluid retention, hypertension, and weight gain. Etoposide is associated with myelosuppression and development of secondary leukemia. Rituximab was well tolerated by our patient and not associated with the development of obvious acute side effects. He did not develop any infusion reactions, including fever or rigors, which have been reported with rituximab. Although he developed a transient period of neutropenia, this side effect was more consistent with the cumulative effects of etoposide than rituximab, which has been linked to late onset neutropenia in certain patients. In support of this possibility, our patient’s bone marrow at the time of neutropenia showed hypocellularity suggestive of generalized myelosuppression and not depletion of myeloid cells or neutrophil maturation arrest, as described previously in rituximab-treated patients.

Our patient received rituximab concurrently with cytotoxic and immunosuppressive therapies. Therefore, it is not possible to delineate his response to any particular agent. His rapid symptomatic improvement may be due to the immunosuppressants. Nevertheless, the pronounced decrease in his EBV viral load is most likely due to the rituximab rather than the other medications. In addition to a rapid improvement in clinical symptoms and decrease in viral load, our patient was in a complete remission after 8 weeks of induction therapy. Interestingly, this result differs from that reported in 2 series of EBV-HLH patients who were treated with similar immunosuppressive regimens but without rituximab, as the majority of these patients did not obtain a complete response after the initial 8-week induction.

Our experience with a single patient yielded encouraging results regarding the addition of rituximab to standard therapy for patients with EBV-HLH. The addition of rituximab seems to be safe and may improve outcome by allowing for omission or reductions in the use of immunosuppressive agents or chemotherapy, thereby reducing the short-term and long-term toxicities of this often devastating condition. On the basis of the positive outcome in our patient, we suggest that further studies be undertaken to investigate this therapy in a larger cohort of EBV-HLH patients.

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REFERENCES

1. Janka G, Imashuku S, Elinder G, et al. Infection- and malignancy-associated hemophagocytic syndromes. Secondary hemophagocytic lymphohistiocytosis. Hematol Oncol Clin North Am. 1998;12:435–444.
2. Imashuku S. Clinical features and treatment strategies of Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis. Crit Rev Oncol Hematol. 2002;44:259–272.
3. Cameron B, Bharadwaj M, Burrows J, et al. Prolonged illness after infectious mononucleosis is associated with altered immunity but not with increased viral load. J Infect Dis. 2006;193:664–671.
4. Kawaguchi H, Miyashita T, Herbst H, et al. Epstein-Barr virus-infected T lymphocytes in Epstein-Barr virus-associated hemophagocytic syndrome. J Clin Invest. 1993;92:1444–1450.
5. Imashuku S, Kuriyama K, Teramura T, et al. Requirement for etoposide in the treatment of Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis. J Clin Oncol. 2001;19:2665–2673.
6. Negasawa M, Okawa H, Yata J. Deleterious effects of high dose gamma-globulin therapy on patients with hemophagocytic syndrome. Int J Hematol. 1994;60:91–93.
7. Ambruoso DR, Hays T, Zwartjes WJ, et al. Successful treatment of lymphohistiocytic reticulosis with phagocytosis with epipodophyllotoxin VP 16-213. Cancer. 1998;35:2516–2520.
8. Fischer A, Virelizier JL, Arenzana-Seisdedos F, et al. Treatment of four patients with erythrophagocytic lymphohistiocytosis by a combination of epipodophyllotoxin, steroids, intrathecal methotrexate, and cranial irradiation. Pediatrics. 1985;76:263–268.
9. Lim WH, Russ GR, Coates PT. Review of Epstein-Barr virus and post-transplant lymphoproliferative disorder post-solid organ transplantation (Review Article). Nephrology (Carlton). 2006;11:355–366.
10. Milone MC, Tsai DE, Hodinka RL, et al. Treatment of primary Epstein-Barr virus infection in patients with X-linked lymphoproliferative disease using B-cell-directed therapy. Blood. 2005;105:994–996.
11. Henter JI, Horne A, Arico M, et al. HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer. 2007;48:124–131.
12. Janka GE. Familial and acquired hemophagocytic lymphohistiocytosis. Eur J Pediatr. 2007;166:95–109.
13. Low PNF, Rascu A, Steininger H, et al. Suppression of HHV-9 viremia by foscarinet in an HIV-infected patient with Kaposi’s sarcoma and HHV-associated hemophagocytic syndrome. Eur J Med Res. 1998;3:461–464.
14. Fisman DN. Hemophagocytic syndromes and infection. Emerg Infect Dis. 2000;6:601–608.
15. Kawada J, Kimura H, Shibat Y, et al. Evaluation of apoptosis in Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis. J Med Virol. 2006;78:400–407.
16. Jordan M, Hildeman D, Kappler J, et al. An animal model of hemophagocytic lymphohistiocytosis (HLH): CD8+ T cells and
interferon gamma are essential for the disorder. Blood. 2004;104:735–743.

17. Sidner RA, Book BK, Agarwal A, et al. In vivo human B-cell subset recovery after in vivo depletion with rituximab, anti-human CD20 monoclonal antibody. Hum Antibodies. 2004;13:55–62.

18. Jabado N, de Graeff-Meeder ER, Cavazzana-Calvo M, et al. Treatment of familial hemophagocytic lymphohistiocytosis with bone marrow transplantation from HLA genetically nonidentical donors. Blood. 1997;90:4743–4748.

19. Imashuku S, Teramura T, Kuriyama K, et al. Risk of etoposide-related acute myeloid leukemia in the treatment of Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis. Int J Hematol. 2002;75:174–177.

20. Kimby E. Tolerability and safety of rituximab (MabThera). Cancer Treat Rev. 2005;31:456–473.

21. Lee JS, Kang JH, Lee GK, et al. Successful treatment of Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis with HLH-94 protocol. J Korean Med Sci. 2005;20:209–214.