Treatment of primary Epstein-Barr virus infection in patients with X-linked lymphoproliferative disease using B-cell directed therapy

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Brief Report: Clinical observations, interventions, and therapeutic trials

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X-linked lymphoproliferative disease (XLP) is a congenital immunodeficiency that is characterized by an abnormal immune response to primary Epstein-Barr virus (EBV) infection. After EBV exposure, affected patients often develop fulminant infectious mononucleosis (FIM), a life-threatening condition marked by the uncontrolled expansion and activation of T and B lymphocytes and macrophages. We hypothesized that the rapid elimination of B cells immediately following EBV exposure might reduce the severity of primary EBV infection in XLP patients. To test this possibility, we administered the anti-CD20 antibody rituximab to 2 patients who presented with acute infection. Following treatment, both patients exhibited a complete resolution of symptoms and no longer demonstrated detectable EBV DNA within circulating lymphocytes. Moreover, neither patient has developed FIM or lymphoma in over 2 years of follow-up. These data suggest that the pre-emptive use of B cell-directed therapy may reduce the morbidity and mortality of primary EBV infection in XLP-affected individuals.
Introduction:

X-linked lymphoproliferative disease (XLP) is a congenital immunodeficiency associated with an inappropriate anti-Epstein-Barr virus (EBV) immune response\(^1\). After primary infection, patients commonly develop a hemophagocytic syndrome known as fulminant infectious mononucleosis (FIM), which is characterized by the polyclonal expansion of EBV-infected B cells and the dysregulated expansion and activation of T cells and macrophages. FIM leads to hepatic and bone marrow (BM) failure and is fatal in over 92% of cases\(^1\).

XLP results from germline mutations in the \(SH2D1A\) gene, which encodes the adaptor protein SAP\(^2\)\(^-\)\(^4\). SAP is expressed in T lymphocytes and natural killer (NK) cells, where it binds to the cytoplasmic domain of SLAM (Signaling Lymphocytic Activation Molecule) and to several structurally related receptors\(^5\). While the roles of SLAM are only beginning to be understood, studies suggest that this receptor functions in a homotypic fashion to bridge adjacent SLAM-expressing cells. SLAM ligation enhances antigen receptor induced T and B cell proliferation and B cell antibody production and modulates T cell cytokine secretion\(^5\). As SLAM expression is rapidly upregulated on activated lymphocytes, the physical interaction between SLAM-expressing EBV-infected B cells and reactive CD4\(^+\) and CD8\(^+\) T cells might guide the development of a normal anti-EBV immune response. Thus, in the absence of functional SAP, SLAM or other SAP-associated receptors might not signal properly, which could compromise the control of EBV-infected B cells and facilitate the secondary expansion of T cells and macrophages. Despite improved understanding of its genetics and biology, XLP remains a fatal disorder curable only by allogeneic hematopoietic stem cell transplantation (HSCT)\(^6\)\(^-\)\(^10\).
There is no evidence that SAP-deficient B cells function differently than normal B cells. Nonetheless, the entry of EBV into B cells is critical for establishing virus infection\textsuperscript{11}. In addition, it remains possible that EBV-infected B cells could promote FIM due to perturbations in SLAM induced responses in SAP-deficient T cells. The anti-CD20 monoclonal antibody rituximab eliminates B cells, a property that has encouraged its use in the treatment of B-cell lymphomas and EBV-associated post-transplantation lymphoproliferative disorders\textsuperscript{12}. Based on these features, we explored its potential to alter the historically fatal outcome of primary EBV infection in XLP patients. Here we describe 2 individuals who presented with acute infection and were successfully treated with regimens including rituximab.

**Methods:**

**Patients**

Patient #1: Patient #1 was a 26-year old Caucasian man who presented with a 3-week history of fever, headache, sore throat and fatigue and a 1-week history of lymphadenopathy. He had 4 siblings who had died from XLP. Previous genetic studies revealed that he carried a germline G383C \textit{SH2D1A} mutation, which was predicted to lead to the substitution of arginine for serine at codon 28 of SAP\textsuperscript{13}. Prior to the current illness, patient #1 had been healthy. He had received monthly intravenous (i.v.) gammaglobulin as prophylaxis against EBV infection.

At presentation, patient #1 showed a mild leukocytosis with a marked lymphocytosis (\textit{Table I}). Large granular lymphocytes and atypical lymphocytes were noted on a peripheral smear. Flow cytometric studies revealed an inverted CD4:CD8 ratio (1:4.4) with NK cells representing 10-
15% of circulating lymphocytes (*not shown*). He also exhibited a mild elevation in liver transaminases. On examination, patient #1 had pharyngeal erythema, cervical adenopathy and a palpable liver edge ~2 cm below the costal margin. Serologic testing for EBV revealed a positive index of 3.26 for IgM antibody to viral capsid antigen. Quantitative EBV-specific polymerase chain reaction (EBV-PCR) revealed a viral load of 18,164 copies of EBV genome/ml of blood.

Patient #2: Patient #2, a 25-year old Caucasian male with 3 brothers who previously died from XLP, was known to harbor a germline *SH2D1A* deletion. He presented with a 4-day history of fever, chills, myalgia, headache, sore throat, nausea and vomiting. Two years previously, patient #2 had undergone splenectomy during the work-up for non-malignant pulmonary nodules and splenomegaly. On examination, patient #2 appeared mildly ill, but had no lymphadenopathy, hepatomegaly or jaundice. Laboratory testing revealed normal peripheral blood counts and minimally elevated transaminases (*Table I*). Serologic testing for acute EBV infection was not performed. Qualitative EBV PCR was positive on the day of presentation. A subsequent quantitative EBV-PCR was obtained on day #8 after initiation of therapy and revealed a viral load of 2,000 copies of EBV genome/ml of blood.

**Treatment Regimens**

Both patients were treated in compliance with Institutional Review Board approved guidelines at the Dana-Farber Cancer Institute and the Hospital of the University of Pennsylvania. Informed consent was obtained in accordance to the Declaration of Helsinki.

Patient #1: On the day of presentation, patient #1 was treated with methylprednisone 1 mg/kg i.v., immunoglobulin 10 g i.v., and ganciclovir 5 mg/kg i.v. every 12 hours X 2 doses, and
rituximab 375 mg/m$^2$ i.v. On day #2, he began oral acyclovir, 800 mg 5 X per day, which was continued for 6 months. He received a second 10 g dose of immunoglobulin on day #4.

**Patient #2:** On the 4$^{th}$ day after presentation, patient #2 was treated with oral prednisone 30 mg twice daily, immunoglobulin 1 g/kg i.v. and oral acyclovir 800 mg 5 X per day. Prednisone was administered for 1 week and tapered over 3 days. Acyclovir was continued for 3 months. On day #5, he received rituximab 375 mg/m$^2$ i.v., which was repeated on day #12 due to persistent atypical lymphocytosis and elevated serum transaminase concentrations.

**Results and Discussion:**

Allogeneic HSCT is the treatment of choice for the prevention of EBV-related morbidity and mortality in XLP$^6$-$^10$. For patients who do not undergo transplantation, acute EBV infection poses a therapeutic challenge as patients rapidly develop FIM. Antiviral therapies, such as acyclovir, ganciclovir and interferon-α have not been successful in preventing or treating FIM$^{15,16}$. While steroids, etoposide and cyclosporine provide symptomatic benefit in some cases of EBV-induced hemophagocytosis$^{17,18}$, FIM remains the most common cause of death for XLP patients$^{1,19}$.

Work from several laboratories has shown that SAP-deficient T and NK cells exhibit defects in cytotoxic function and IFNγ production, factors that might interfere with clearance of EBV-infected B cells$^5$. As persistence of these cells might subsequently promote the proliferation and activation of reactive T cells and macrophages, we hypothesized that the rapid eradication of EBV-infected B cells immediately after exposure might reduce viral burden and prevent the secondary hemophagocytic syndrome that typifies FIM. Because anti-CD20 immunotherapy
with rituximab leads to the elimination of more than 80% of circulating B cells within days\textsuperscript{20}, we developed individualized therapies centered around this medication for 2 XLP patients presenting with acute EBV infection. These regimens varied slightly based on the preferences of treating oncologists, who were from different institutions. However, in both regimens, rituximab was combined with steroids to suppress T cell activation. Based on historical approaches, both regimens also included more traditional anti-viral agents, such as acyclovir and ganciclovir.

Following initiation of treatment, both patients demonstrated a rapid reduction of B cells to 1% or less of circulating lymphocytes (not shown). This decrease in B cell number was associated with a decline in peripheral blood leukocyte-associated EBV DNA to levels below the limit of detection (Figure 1). In both patients, clinical improvement occurred within 24-48 hours, as was evident by a return to normal body temperature and disappearance of malaise and fatigue. Notably, neither patient developed hemophagocytosis. Despite rapid improvement in clinical and virologic features, certain laboratory parameters, such as elevations in transaminases, white blood cell count and percentage of atypical lymphocytes required 4-16 weeks to return to baseline levels (Figure 1 and not shown). When compared with the prolonged convalescence seen in adult immunocompetent individuals with mononucleosis, the rapid recovery of these patients suggests that rituximab exerts a positive effect on the course of acute EBV infection.

Rituximab reduces the number of circulating B cells for up to 6 months or longer\textsuperscript{20}. Although lymphomas are known to occur in EBV-positive and EBV-negative XLP patients, the development of lymphoma occurs earlier in EBV-infected individuals\textsuperscript{21}. We do not know whether the reduction in B cell number that resulted following rituximab therapy will prevent the
development of lymphoma in these treated patients. However, neither patient has developed lymphoma in more than 24 months of follow-up. Interestingly, surveillance monitoring in patient #1 revealed the onset of hypogammaglobulinemia at 5 months post-treatment with rituximab, and re-emergence of detectable EBV DNA at 14 months following treatment (not shown). Thus, rituximab may not prevent the natural progression of hypogammaglobulinemia or lead to long-term control of EBV infection in certain XLP patients. As neither patient has undergone testing for EBV-specific T or B cell immunity since treatment with rituximab, it is not possible to discern how this therapy will influence their long-term immunological response to EBV.

Rituximab induces a rapid improvement in the clinical symptoms associated with acute EBV infection in patients with XLP. We recommend that rituximab be considered as part of the initial management for XLP patients following primary EBV exposure. Furthermore, the pre-emptive use of rituximab may benefit the management of other disorders associated with EBV-induced hemophagocytosis, such as the familial or acquired hemophagocytic lymphohistiocytoses.

**Acknowledgements:** We would like to thank the patients who participated in this investigation.
References:


Table 1:

Laboratory findings in the 2 XLP patients at presentation with primary EBV infection

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<tr>
<th></th>
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Figure Legends:

Figure 1: Changes in liver transaminases and EBV DNA levels are shown for patients #1 and #2 following treatment with rituximab. The time line below each figure indicates the time points at which quantitative EBV PCR was performed and the values that were obtained. The arrows above the time lines indicate when rituximab was administered.
Figure 1.

**Patient #1**

![Graph showing AST and ALT levels over weeks for Patient #1 with EBV viral load data.](image1)

**Patient #2**

![Graph showing AST and ALT levels over weeks for Patient #2 with EBV viral load data.](image2)
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