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Successful Treatment of X-Linked Lymphoproliferative Disease (XLP) with Anti-CD20 Monoclonal Antibody (Rituximab) Followed by Mismatched Unrelated Cord Blood Transplantation

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Abstract A 4-year-old boy with X-linked lymphoproliferative disease (XLP) developed life-threatening acute lymphoproliferative crisis and failed to respond to conventional treatment of dexamethasone and etoposide. With the knowledge that uncontrolled alloreactive cytotoxic T-cell responses triggered by EBV-transformed B cells is the main cause of XLP, anti-CD20 monoclonocal antibody (Rituximab) which directed against B lymphocytes was use to damp down the patient's dysregulated immune response. He responded well to this novel approach and entered into complete remission with this treatment. His inherited immuno-deficient genetic defect was subsequently corrected by unrelated cord blood transplantation.

Key words Anti-CD20 monoclonal antibody; Cord blood transplantation; Rituximab; X-linked lymphoproliferative disease

Introduction

In X-linked lymphoproliferative disease (XLP), also known as Duncan's disease, there is a failure in controlling the proliferation of cytotoxic T cells triggered by Epstein-Barr virus (EBV) infection. Patients with this disorder usually remain asymptomatic until they are infected by EBV, which usually occurs within the first five years of age. The commonest form of presentation (occurring in 75 percent of cases) is severe infectious mononucleosis, which is fatal in 80 percent of patients. The high mortality is primarily due to extensive liver necrosis caused by activated cytotoxic T cells.[1]

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The diagnosis of XLP is mainly based on clinical criteria. It requires two or more maternally related males manifesting one or more of the following characteristic phenotypes: fulminant infectious mononucleosis (IM), malignant lymphoma or lymphoproliferative disease, and dysgammaglobulinemia.[2] Most boys who survive EBV infection have global cellular immune defects, and lymphomas, aplastic anemia, or hypogammaglobulinemia will ultimately develop.

The defective gene in X-linked lymphoproliferative disease is located at Xq25 and encodes an adapter protein found in T cells and natural killer cells known as SLAM-associated adaptor protein (SAP). SAP couples with downstream signaling molecules to a cell surface protein known as signaling lymphocyte activation molecule (SLAM) found on the membrane surfaces of T and B cells. SLAM is an unusual membrane protein that serves both a growth-promoting molecule and a receptor for itself. SAP controls signaling via the SLAM family of surface receptors and plays crucial roles in T-cell and antigen presenting cell interactions during viral infections.[3-5]

Most conservative treatment modalities designed for the XLP including chemotherapy, antiviral agents, high-dose intravenous immunoglobulin and interferon (alpha and gamma) have been unsuccessful. Allogeneic bone marrow transplantation (BMT) is the only therapy with a proven

curative potential currently. Thirteen patients with X-linked lymphoproliferative disease have been reported to undergo HLA-matched haematopoietic stem cell transplants, and approximately half of them showed durable remission with no recurrent of related complications.[6-8] Here, we report a 4 years boy with X-linked lymphoproliferative disease in whom his life-threatening acute lymphoproliferative crisis was controlled with anti-CD20 monoclonal antibody (rituximab) and his XLP was subsequently corrected by mismatched unrelated cord blood transplantation.

Case Report

The patient was diagnosed as XLP based on positive family history, compatible clinical phenotype and laboratory findings. His elder brother died at the age of 2-year-old with the diagnosis of 'haemophagocytic lymphohistiocytosis'. He presented at the age of 3 years with high fever, generalised lymphadenopathy, gross hepatosplenomegaly and later aplastic anaemia. He did not respond to multiple broad spectrum antibiotics and antivirals such as high dose acyclovir and foscarnet. Because of his dysfunction immune system and aplastic anaemia, he was complicated by repeated episodes of documented septicemia by gram positive and negative organisms including *Streptococcus pneumoniae*, *methicillin-resistant coagulase-negative staphylococcus*, *Acinetobacter baumannii* and *Klebsiella pneumoniae* respectively. Due to the poor general state and respiratory failure induced by these infections, he required intensive care with mechanical ventilation. His laboratory findings were summarised in Table 1 and Figure 1.

He was treated empirically with dexamethasone and

Table 1 Virology profile, bone marrow PCR study and SAP protein analysis

Test		**Results**
EBV profile	Anti-VCA Ig G and Ig M	Positive
	Anti-EA	Positive
	Anti-EBNA	Negative
	Anti-VCA	Positive
	EBV PCR (plasma)	Positive
HHV 6 and 7 profile	HHV 6 PCR (plasma)	Positive
	HHV 7 PCR (plasma)	Negative
CMV profile	CMV PCR and CMV pp65 antigen	Negative
Bone marrow examination	Trephine biopsy	Hypocellular marrow with atypical lymphoid infiltration
	PCR analysis	No clonal TCR or Ig H gene rearrangement No clonal immunoglobulin heavy chain or T-cell receptor gene rearrangement
	In-situ hybridisation study for EBER	Showed positivity mainly in the population of large mononuclear cells; these cells most probably correlate with B-cell population Compatible with EBV-related B-cell lymphoproliferative disorder
Immune function	Ig G / Ig A / Ig M	1194 / 78 / 784 mg/dl
	Lymphocyte subset	See Figure 1
	Lymphocyte proliferation test	Normal Lymphocyte response to PHA, Con A and PWM
SAP protein analysis	SAP gene expression	No SAP mRNA and genomic DNA were detected for the patient. A large deletion in patient's X chromosome is suspected. The deleted region, of minimum of 25 kb (from exon 1 to exon 4), included the whole SAP gene coding sequence.
	Immunoflourescence staining (flow cytometry)[9]	Absence of SAP protein expression in T cell blast

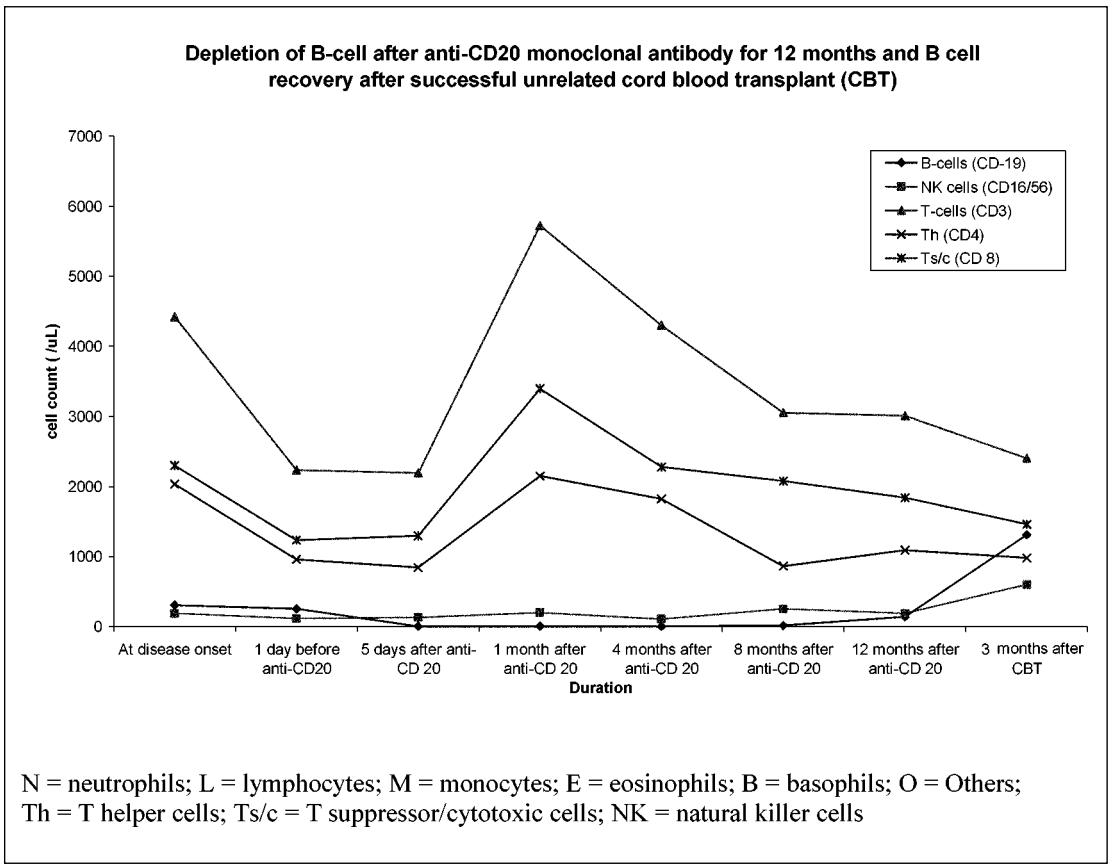

Figure 1 Serial change of lymphocyte subset profile before and after anti-CD20 monoclonal antibody.

etoposide (VP-16) (modified from the protocol for haemophagocytic lymphohistiocytosis HLH-94) but the response was unsatisfactory. His condition remained critical and based on the encouraging experience of using anti-CD20 monoclonal antibody (rituximab) in post-transplant lymphoproliferative disease (PTLD), which is also an EBV-driven malignant lymphoproliferative disease in immunocompromised host, we finally decided to use similar approach for this critically ill child.[9,10] Anti-CD20 (Rituximab) 385 mg/m^2/dose weekly for 4 weeks were given. His condition improved dramatically after the treatment. Fever subsided within one week with resolving hepatosplenomegaly and lymphadenopathy. Serial lymphocyte subset showed almost absence of B cell population due to the effect of anti-CD20 for nearly 12 months.

Since he has no HLA-matched sibling in the family, he was given maintenance therapy whilst searching for HLA-matched unrelated donor. The maintenance therapy consisted of monthly intravenous immunoglobulin (IVIG) infusion; oral acyclovir and co-trimoxazole prophylaxis; VP-16 (150 mg/m^2 every 2 week); pulse dexamethasone 10 mg/m^2 daily for 3 days every 2 weeks and low dose oral cyclosporine (2 mg/kg) twice daily. He received this maintenance therapy for 12 months and remained clinically stable with no flaring-up of his disease.

Due to the difficulty in finding a compatible matched donor, he finally proceeded to a 2-antigen mismatched unrelated cord blood transplant (one at HLA-A locus and the other at HLA-DR locus, also sex mismatched). The conditioning regimen consisted of busulphan 5 mg/kg from day -9 to -6; cyclophosphamide 50 mg/kg from day -5 to day -2; Anti-thymocyte globulin (ATG-horse) 30 mg/kg from day -5 to day -2. The cord blood contained 3.53 x10^7 nucleated cells per kg recipient body weight and CD34 positive cells were 35.7 x 10^4 per kg recipient body weight. Cyclosporine and methotrexate were given as graft-versus-host disease (GvHD) prophylaxis. An absolute neutrophil count of 0.5 x10^9/L was reached at 32 days post transplant. Full donor engraftment was documented at 14, 31 and 63 days post transplant as demonstrated by 100% donor female cells in peripheral blood by FISH. He was complicated by

grade 2 acute GvHD involving skin which was readily controlled with low dose steroid. The major post-transplant complications were severe haemorrhagic cystitis requiring formalin bladder irrigation and cyclosporine related encephalopathy requiring change of immunosuppressant. He recovered from these complications with no obvious long term consequence. Lymphocyte subset profile at post transplant showed recovery of B-cell population. SAP protein analysis showed the presence of SAP protein indicating correction of underlying immune defect. Serial EBV, HHV 6 and HHV 7 PCR showed no reactivation of these viruses. We also noticed the interesting phenomenon of surge of fetal haemoglobin (Hb-F) level during cord blood engraftment (Figure 2). The surge is similar to that observed in thalassaemia major patients undergoing cord blood transplant as reported by us previously,[11] which indicated engraftment in the context of cord blood transplant. He is now well with no medication and already resuming his normal activities at 40 months post cord blood transplant.

Discussion

Here, we reported a patient with XLP whose fulminant EBV infection was successfully controlled with anti-CD20 antibody. He subsequently underwent mismatched unrelated CBT and is currently cured. It was well known that XLP patients are susceptible to EBV virus infection. In this patient, we demonstrated that his clinical course might also be affected by other herpes family of viruses such as HHV 6 and HHV 7. This was demonstrated by the positive PCR for these viruses in the plasma at the disease onset.

Usually, the mortality is extremely high once patient with XLP is infected with EBV. During the acute state, transplant cannot be preformed if the patient's condition fails to be stabilised. The use of VP16, prednisolone, IVIG or acyclovir often cannot induce 'remission' for most of the cases such as ours. Based on the hypothesis that uncontrolled alloreactive cytotoxic T-cell responses triggered by EBV-transformed B cells is the basic pathophysiology of XLP, we used a novel approach of applying anti-CD20 antibody to damp down the patient's immune response and it was proven to be helpful.

Anti-CD20 (Rituximab) is a humanised monoclonocal antibody directed against the specific CD20 antigens found on B lymphocytes plasma membrane. It has been approved by the FDA for the treatment of lymphoma and post-transplant lymphoproliferative diseases. Its uses in several autoimmune diseases have recently been explored. The use of anti-CD20 monoclonal antibody in this patient serves dual purposes. Firstly, his body was mounting an intense but futile immune response to the EBV-infected B

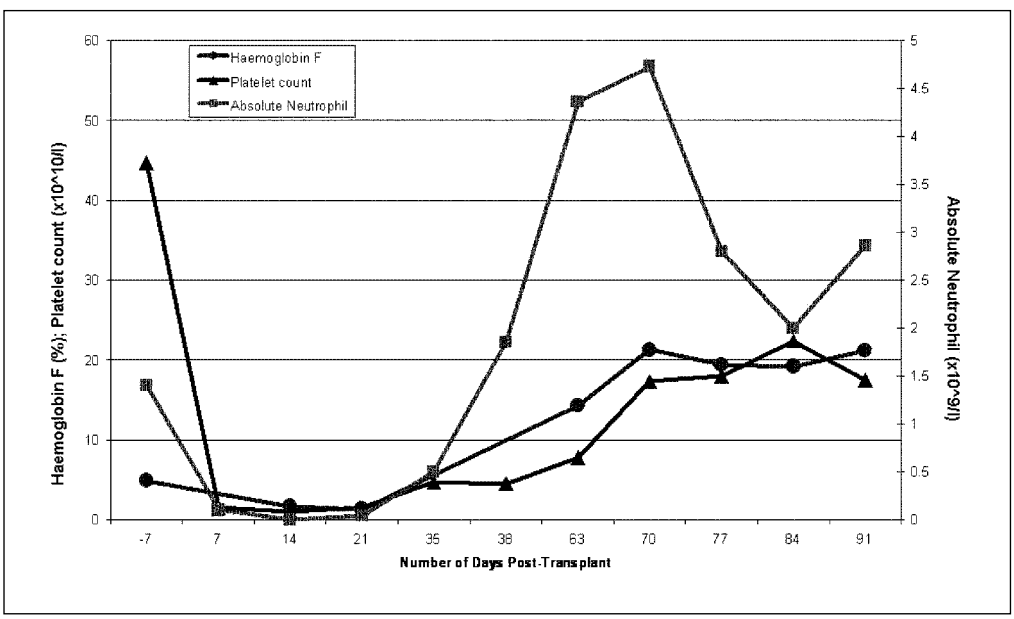

Figure 2 Surge of fetal haemoglobin level coinciding with cord blood engraftment.

lymphocytes, this leads to the clinical phenotype of lymphoproliferative disease and aplastic anaemia. Therefore, depleting the EBV-infected B lymphocytes so as to remove the upstream stimulus of this futile exaggerated immune response will be helpful. Secondly, it was well known that some patients with XLP may eventually evolve into lymphoma in long term. Therefore, anti-CD20 may serve as pre-emptive treatment to prevent clonal evolution of these EBV-transformed B lymphocytes. As we expected, he successfully achieved remission and improved dramatically after the treatment. However, prolonged duration of severe B lymphopenia is a consequence of this treatment. Paradoxically, the absence or near absence of B cell may actually be beneficial for him as there would be no EBV-infected B cells available to trigger another fulminant immune damage subsequently. Afterwards, we were able to keep him stable without major flare-up over a 12 month period. Maintenance therapy of regular VP-16, dexamethasone, cyclosporine and IVIG were given whilst we were searching for compatible unrelated donor. As this is a rare condition, only large scale multi-centre study can help us to further evaluate the role of monoclonal anti-B cell antibodies in XLP patients with acute lymphoproliferative disease.

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