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■ Case report ■

Unrelated stem cell transplantation after reduced-intensity conditioning plus rituximab for Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis with CNS involvement

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= Abstract =

Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis (EBV-HLH) with central nervous system (CNS) involvement is usually fatal unless stem cell transplant (SCT) is offered. However, SCT with conventional intensity conditioning is associated with high transplant-related mortality. We describe our experience with unrelated SCTs after reduced-intensity conditioning (RIC) for patients with EBV-HLH with progressive CNS disease. This approach was associated with minimal toxicities and might be an effective option in patients with EBV-HLH with progressive CNS disease. Moreover, the addition of rituximab to RIC appears to be safe and effective in suppressing EBV in the patients with EBV-HLH. (Korean J Pediatr 2009;52:725-729)

Key Words : Unrelated stem cell transplantation, Reduced-intensity conditioning, Rituximab, Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis, CNS involvement

Introduction

Hemophagocytic lymphohistiocytosis (HLH) is an unusual immunological disorder in infants and children. Patients with HLH present with a potentially fatal syndrome of hyperimmunity and severe inflammation associated with cytopenia, and variable liver or central nervous system (CNS) abnormalities[1]. HLH can be either a familial or secondary disorder and the two can be difficult to distinguish. Secondary HLH is associated with severe infections, malignancies or rheumatic diseases. Epstein-Barr virus associated HLH (EBV-HLH) usually has more rapid clinical course with severe clinical features[2]. The only cure for familial HLH is allogeneic stem cell transplantation (SCT). Also SCT should be considered in secondary HLH when the disease persists or relapses although chemotherapy and/or immunotherapy may be effective in achieving the clinical remission of symptoms initially[3]. Previous studies[3-6] on SCT with conventional intensity conditioning have shown that the transplant-related mortality (TRM) rate is high, mostly from noninfectious pulmonary toxicity and veno-occlusive disease (VOD). However, results of SCTs with reduced-intensity conditioning (RIC) are encouraging recently.

We describe our experience with unrelated SCTs after RIC plus rituximab for patients having EBV-HLH with progressive CNS disease. The conditioning regimen consisted of fludarabine 30 mg/m^2 daily for 5 days, melphalan 140 mg/kg for 1 day, and alemtuzmab 0.2 mg/kg daily for 5 days[7, 8] Addition of rituximab, 300 mg/m^2 was used to prevent EBV reactivation on Day-1.

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Case report

1. Case 1

A previously healthy 3-year-old boy was admitted because of fever, abdominal pain and vomiting of 7 days duration. The physical examination was notable for hepatosplenomegaly. Laboratory evaluation showed the followings: white blood cell count, 3,300/μL; hemoglobin level, 11.6 g/dL; pla-

telet count, 68×10^9/L; triglycerides, 185 mg/dL (reference range: 30-86 mg/dL); fibrinogen 215 mg/dL (200-400 mg/dL); ferritin 653 ng/mL (7-140 ng/mL). Antibodies against EBV such as viral capsid antigen (VCA), early antigen (EA), EB nuclear antigen (EBNA) were negative and the bone marrow (BM) examination showed increased numbers of histiocytes without hemophagocytosis. There was no family history of HLH or immunological disorders and there was no perforin mutation noted. Dexamethasone and cyclosporine were administered as he showed pleural effusion and dyspnea. One month later despite on cyclosporine, the fever and pleural effusion reappeared and a repeat BM revealed increased hemophagocytic histiocytes. Etoposide was added per the HLH-2004 protocol and the symptoms resolved. Two months after completion of the initial therapy of the HLH 2004 protocol, the patient presented with a simple partial seizure, left facial palsy and gait disturbance. The brain CT (Fig. 1A) showed multiple irregular ring enhancing nodules and calcification at the corticomedullary junction of the cerebral hemispheres and right cerebellar hemisphere[9]. The cerebrospinal fluid (CSF) exam revealed 11 white cells/L and protein 136 mg/dL. The patient was suspicious of having multiple brain abscesses seen in immunocompromised hosts. Empiric treatment with amphotericin, pyrimethamin and sulfadiazine was given for two weeks. However, a left hemiparesis was aggravated and the size of the lesions on follow-up brain CT (Fig.1B) scanning increased. A biopsy of the brain lesions showed lymphohistiocytosis associated with EBV infection, as evidenced by positive *in situ hybridization* for EBV (Fig. 1C). The patient was treated again with the initial therapy of HLH-2004 along with four doses of intrathecal methotrexate (MTX) and had gradually improved over the next several weeks. Six months after the onset of the CNS signs, a two-allele (2/8) mismatched unrelated BM transplantation was performed.

The GvHD prophylaxis consisted of tacrolimus with a short course of MTX. The days to engraftment for neutrophils and platelets were 15 and 22, respectively. Limited chronic GvHD involving the skin developed. An escalating doses of tacrolimus resulted in complete resolution of GvHD. The brain CT (Fig. 1D), at 32 months post-transplantation, revealed improvement of the lesions noted before the transplantation. Persistent mixed chimerism with variable donor predominance (51-94%) was found. However, there was no evidence of HLH or EBV reactivation or GvHD over 34 months following the transplant. The patient had normal psychomotor development with a Lansky performance score of 100%.

2. Case 2

A 7-month-old girl was diagnosed with HLH at another hospital. She presented with fever, seizures, quadriparesis and the CSF exam showed 110 white cells/L and a protein of 140 mg/dL. The brain MRI revealed multifocal hyperintense lesions in the left temporal lobe and findings of meningoencephalitis (Fig. 2A). EBV IgG and IgM antibodies against VCA, EA, EBNA were negative. The patient was treated according to the HLH-2004 protocol. During the continuation therapy on the HLH-2004 protocol, the patient relapsed and the brain lesions on MRI worsened (Fig. 2B). The initial therapy based on the HLH-2004 was started again and the patient was referred to our hospital. EBV antibodies against VCA and EBNA IgG antibodies became positive. There was no family history of HLH or other immunological disorders and there was no perforin mutation.

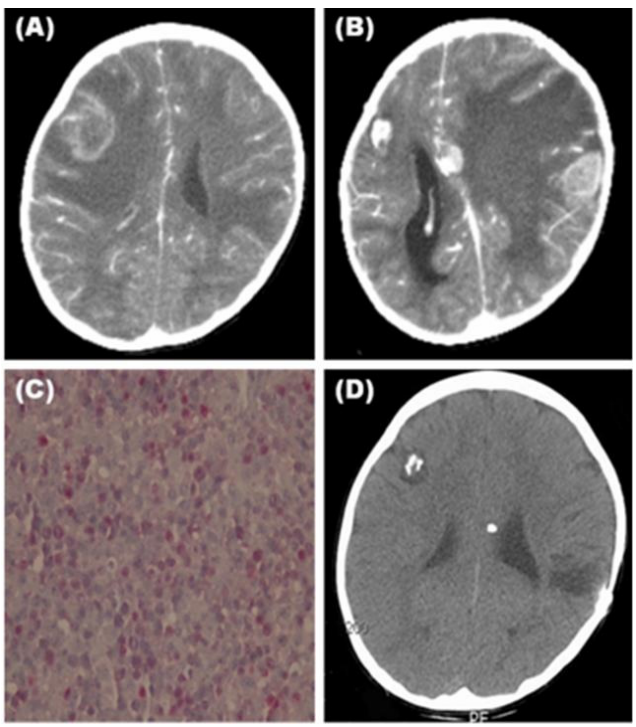

Fig. 1. (A) Brain CT at the onset of seizures and left hemiparesis shows multiple irregular ring enhancing nodules. (B) Follow up brain CT after aggravation of symptoms shows an increase in the size of the enhancing nodules with aggravated perilesional edema and a midline shift to the right. (C) The brain biopsy revealed many foamy histiocytes and atypical lymphocyte infiltration associated with (+) in situ hybridization for EBV (H&E stain; ×400). (D) The brain CT at 21 months post-transplant reveals improvement of the lesions.

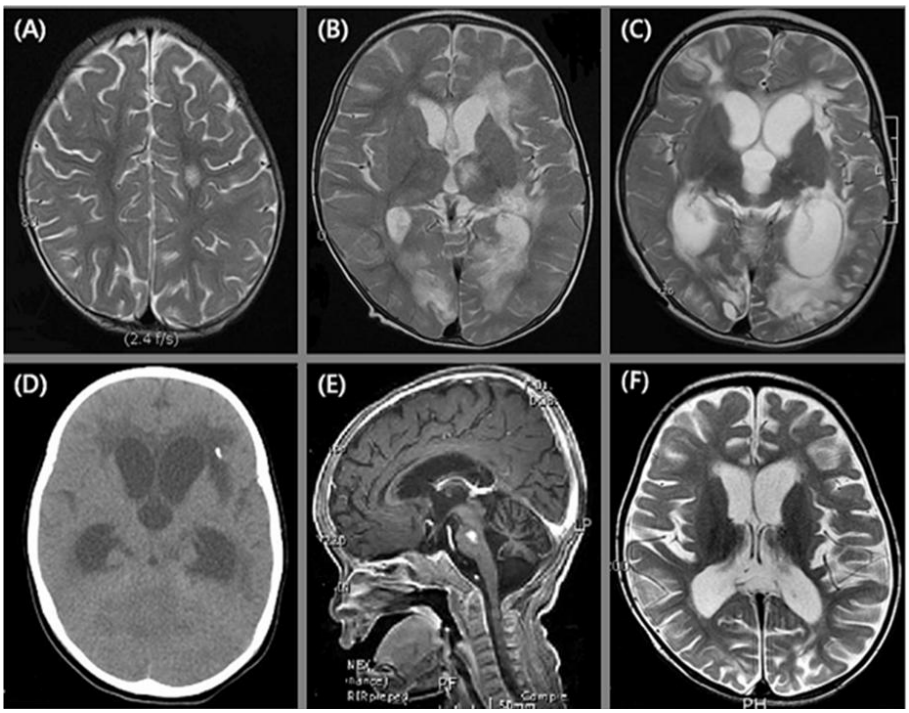

Fig. 2. (A) At diagnosis, the MRI shows multifocal hyperintense leisions at the left temporal lobe and splenium of the corpus callosum. (B) At relapse, the MRI shows hydrocephalus and aggravation of the hyperintense lesions in both hemispheres and cerebellum, especially in the left basal ganglia, thalmus, brain stem and cerebellum. (C, D) Pretransplant brain MRI and CT show hydrocephalus and patchy hypersignal leisions in the periventricular and subcortical white matter of the cerebral and cerebellar hemispheres, and pons with small subcortical calcifications. (E, F) Five months post-transplant, the MRI shows nodular enhancing lesions in the brain stem, cerebellar hemispheres and both occipital lobes with improvement of the lesions noted previously.

Two weeks prior to the SCT, external ventricular drainage was performed due to hydrocephalus (Fig. 2C, D). Three months after the relapse, the patient underwent a reduced intensity 5/6 matched unrelated cord blood transplantation. GvHD prophylaxis consisted of tacrolimus and mycophenolate mofetil. Engraftment was uneventful and the days to engraftment for neutrophils and platelets were 24 and 26, respectively. Mixed chimerism with donor predominance (75-80%) was persistent. The CNS lesions were decreased (Fig. 2E, F) and the neurological impairment gradually improved after transplantation. However, acute GvHD of Grade IV involving the skin and gut developed, but it was refractory to steroid or antithymocyte globulins. The patient died of chronic GvHD and invasive candidiasis 6 months after the transplantation.

Discussion

SCT should be considered in the familial HLH or persistent, recurring form of secondary diseases[1, 3]. A recent SCT series[3-6] with a myeloablative conditioning regimen including busulfan, cyclophosphamide, and etoposide, with or without antithymocyte antibodies, reported the event free survival of 58-64% but the high TRM of 21-35%, especially in unrelated donor SCTs. VOD and pneumonitis contributed to the TRM and accounted for half of all early deaths in one study[3]. Primary non-engraftment occurred in 9-22% and secondary or late rejection in 0-14% in a myeloablative SCT series[3-5]. The reason for a higher TRM rate was unclear, but might be explained in part by factors related to the disease itself; there might be inadequate immunosuppression to maintain control of the aberrant host immune system by traditional myeloablative conditioning regimens.

Cooper et al[7, 8] reported on 25 patients with HLH treated by RIC SCT consisting of fludarabine, melphalan and serotherapy with alemtuzmab. Twenty-one (84%) of these patients were alive and well with disease remission at a median of 36 months after the SCT. There were four TRMs (16%)

including pneumonitis in three and hepatic rupture in one case. Six of the twenty-one survivors developed stable mixed chimerism and no patient rejected their graft or relapsed.

Our two patients underwent mismatched unrelated SCTs with RIC. They showed uneventful engraftment and no development of VOD. Despite mixed chimerism, they were free of HLH. Case 2 died of GvHD and fungal infection, but was in complete remission with regard to the HLH.

CNS involvement is common in HLH and is highly variable in its presentation. In addition, CNS reactivation may occur during therapy. One study[10] reported either neurological symptoms and/or abnormal CSF at diagnosis in 63% of cases, and abnormal neuroradiological findings in 30%. The common radiological findings were generalized cerebral atrophy, periventricular white matter lesions, demyelination, necrotic areas, and multiple enhancing bilateral hemispheric lesions with edema[11, 12], as observed in our cases. Patients who present with either clinical neurological manifestations or abnormal neuroradiological findings before SCT have been identified as having a poorer prognosis[5]. Our cases had abnormal MRI findings and abnormal CSF findings either at the time of diagnosis or relapse. In Case 1, the CT and MRI findings of multiple ring enhancing parenchymal lesions mimicking abscesses or other nonspecific findings, disappeared[9] after initial therapy with the HLH-2004 protocol, but calcifications remained. In Case 2, the CNS lesions decreased and the neurological impairment gradually improved after transplantation.

Although the prognosis for secondary HLH is often better than inherited HLH, EBV-HLH has been associated with a high morbidity and mortality[2]. Most reports describing successful outcomes for patients with EBV-HLH have used immunosuppressive medications to inhibit overactive T and NK cell responses, in conjunction with chemotherapeutic agents, to target dividing lymphocytes and mononuclear phagocytes[2]. The use of etoposide is critical for securing long-term survival in patients with EBV-HLH but is associated with the development of secondary leukemias[2]. Rituximab, a humanized anti-CD20 monoclonal antibody that targets mature B cells, has become an attractive option for reducing the viral burden in a number of EBV-mediated diseases including the EBV-induced lymphoproliferative disorder[13] and has been reported to be effective in EBV-associated HLH[14]. In the cases reported here, we used rituximab to prevent EBV reactivation and performed RIC SCT without etoposide, and EBV reactivation was not observed.

In conclusion, RIC SCT for HLH was associated with prompt engraftment and minimal toxicity. HLH with progressive CNS disease may be cured after RIC SCT. Moreover, the addition of rituximab to RIC appears to be safe and effective in suppressing EBV reactivation. However, more patients are needed to determine the efficacy and feasibility of this regimen in patients with HLH, especially in the cases with CNS involvement.

한 글 요 약

저강도 전처치와 rituximab 후 타인 조혈모세포 이식을 시행한 중추신경계를 침범한 Epstein-Barr 바이러스 관련 혈구포식 림프조직구증

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중추신경계의 침범을 동반한 Epstein-Barr 바이러스 관련 혈구포식 림프조직구증은 조혈모세포이식을 시행하지 않으면 예후가 좋지 않다. 또한, 고식적인 강도의 전처치시 이식관련 사망률이 높다. 따라서 저자들은 진행성 중추신경계 침범을 보인 Epstein-Barr 바이러스 관련 혈구포식 림프조직구증 2례에서 저강도 전처치와 rituximab 후 타인 조혈모세포이식을 시행하여 치료하였기에 이를 보고하고자 한다.

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