was performed with 7 humanized NOG mice that were not infected with EBV; 4 mice were given OKT3 antibody, and 3 were not. The result indicated that all mice in both groups survived the observation period of 150 days, and no difference could be seen between them, excluding the possibility that OKT3 has its own toxicity (Figure 1*C*). In accordance with this result, quantification with real-time polymerase chain reaction indicated that the level of EBV DNA in the peripheral blood was consistently higher in the OKT3-treated mice than in control mice (Figure 1*D*). Because $CD8^+$ cytotoxic T cells are considered to play a central role in the immune response to EBV, we next examined the effect of an antibody (B9.11) specific to the CD8 molecule [14]. Figure 1*E* shows the survival curves of EBV-infected humanized NOG mice that were either treated with B9.11 or not, and the log-rank test indicated that those mice treated with the antibody had a significantly reduced life span after EBV infection ($P < .05$).

To test whether T cells isolated from EBV-infected mice have the ability to suppress transformation of autologous B cells by EBV, we used the transformation regression assay. Mononuclear cells isolated from the spleen of humanized NOG mice were inoculated with EBV and were cocultured with $CD8^+$ T cells isolated from the spleen of either EBV-infected or uninfected mice. Table 1 shows the number of $CD8^+$ T cells required to inhibit the outgrowth of EBV-infected cells in 50% of the wells (50% regression dose). These data indicate that $CD8^+$ T cells isolated from EBV-infected mice, but not those from uninfected mice, could suppress the outgrowth of transformed lymphoblastoid cell line.

Discussion. Humanized mouse technology has been successfully used to reproduce human immune responses to certain viruses that cannot infect ordinary mice [6–8, 10]. However, these studies have yet to provide evidence that immune responses induced in these mice are actually involved in the control of viral infections. This was an important issue when we considered the possibility of using humanized mice as a tool to evaluate immunological therapies and prophylaxes to viral infections.

Humanized NOG mice in later stages of reconstitution that contained high numbers of T cells had significantly longer life spans after EBV infection, compared with those in earlier stages that lacked significant differentiation of T cells. In accordance with this result, treatment of EBV-infected humanized NOG mice with OKT3, which can deplete $CD3^+$ T cells, significantly reduced their life span. Furthermore, similar antibody-mediated reduction in the number of $CD8^+$ T cells also reduced the life span of infected humanized NOG mice. Thus, our results strongly suggest that human T cells that develop in humanized NOG mice contribute to their resistance to EBV infection. More-direct evidence of immunological control of EBV infection was obtained by the transformation regression assay, which has been used as one of the most reliable methods to quantify EBV-specific cytotoxic T cells. This assay clearly indicated that $CD8^+$ T cells isolated from EBV-infected mice can suppress EBV-induced transformation of autologous B cells. A study is underway to identify an epitope that can be recognized by a $CD8^+$ T cell clone.

To our knowledge, these results are the first evidence that immune responses induced in humanized mice are actually involved in an effective control of viral infection. It is thus suggested that the NOG mouse model of EBV infection is a useful tool to evaluate candidate vaccines and immunological therapies for EBV infection.

After the initial submission of this report, Strowig and others published a work containing similar findings, namely higher EBV load and lymphoproliferative disorder in humanized mice after T cell depletion [15].

Acknowledgments

We thank Motohiko Okano, for advice in regression assay; Miki Mizukami, Ken Watanabe, and Miki Katayama, for technical assistance; Yuko Tatsumi and Tomomi Hakuya, for secretarial assistance; and Tokyo Cord Blood Bank, for supplying cord blood.

References

1. Rickinson AB, Kieff E. Epstein-Barr virus. In: Knipe DM, Howley PM, eds. Fields virology. 5th ed. Vol. 2. Philadelphia: Lippincott Williams & Wilkins, **2007**:2655–700.
2. Kieff E, Rickinson AB. Epstein-Barr virus and its replication. In: Knipe DM, ed. Fields virology. 5th. ed. Vol. 2. Philadelphia: Lippincott Williams & Wilkins, **2007**:2603–54.
3. Hislop AD, Taylor GS, Sauce D, Rickinson AB. Cellular responses to viral infection in humans: lessons from Epstein-Barr virus. Annu Rev Immunol **2007**; 25:587–617.
4. Babcock GJ, Decker LL, Volk M, Thorley-Lawson DA. EBV persistence in memory B cells in vivo. Immunity **1998**; 9:395–404.
5. Ito M, Hiramatsu H, Kobayashi K, et al. NOD/SCID/gamma(c)(null) mouse: an excellent recipient mouse model for engraftment of human cells. Blood **2002**; 100:3175–82.
6. Yajima M, Imadome KI, Nakagawa A, et al. A new humanized mouse model of Epstein-Barr virus infection that reproduces persistent infection, lymphoproliferative disorder, and cell-mediated and humoral immune responses. J Infect Dis **2008**; 198:673–82.
7. Melkus MW, Estes JD, Padgett-Thomas A, et al. Humanized mice mount specific adaptive and innate immune responses to EBV and TSST-1. Nat Med **2006**; 12:1316–22.
8. Traggiai E, Chicha L, Mazzucchelli L, et al. Development of a human adaptive immune system in cord blood cell-transplanted mice. Science **2004**; 304:104–7.
9. Watanabe S, Ohta S, Yajima M, et al. Humanized NOD/SCID/IL2Rgamma(null) mice transplanted with hematopoietic stem cells under nonmyeloablative condition show prolonged lifespans and allow detailed analysis of human immunodeficiency virus type 1 pathogenesis. J Virol **2007**; 81:13259–64.
10. Watanabe S, Terashima K, Ohta S, et al. Hematopoietic stem cell-engrafted NOD/SCID/IL2Rgamma null mice develop human lymphoid

systems and induce long-lasting HIV-1 infection with specific humoral immune responses. Blood **2007**; 109:212–8.
11. Moss DJ, Rickinson AB, Pope JH. Long-term T-cell-mediated immunity to Epstein-Barr virus in man. I. Complete regression of virus-induced transformation in cultures of seropositive donor leukocytes. Int J Cancer **1978**; 22:662–8.
12. Okano M, Purtilo DT. Simple assay for evaluation of Epstein-Barr virus specific cytotoxic T lymphocytes. J Immunol Methods **1995**; 184:149–52.
13. Dessureault S, Shpitz B, Alloo J, et al. Physiologic human T-cell responses to OKT3 in the human peripheral blood lymphocyte-severe combined immunodeficiency mouse model. Transplantation **1997**; 64: 811–6.
14. Kobayashi E, Kawai K, Ikarashi Y, Fujiwara M. Mechanism of the rejection of major histocompatibility complex class I-disparate murine skin grafts: rejection can be mediated by CD4+ cells activated by allo-class I + II antigen in CD8+ cell-depleted hosts. J Exp Med **1992**; 176: 617–21.
15. Strowig T, Gurer C, Ploss A, et al. Priming of protective T cell responses against virus-induced tumors in mice with human immune system components. J Exp Med **2009**; 206:1423–34.

Fatal degeneration of specialized cardiac muscle associated with chronic active Epstein–Barr virus infection

Daiichiro Hasegawa,[1] Michiko Kaji,[1] Hiroki Takeda,[1] Keiichiro Kawasaki,[1] Hironobu Takahashi,[2] Hiroshi Ochiai,[3] Tomohiro Morio,[3] Yasuhiro Omori,[4] Hiroshi Yokozaki[4] and Yoshiyuki Kosaka[1]

[1]*Department of Hematology and Oncology, Kobe Children's Hospital,* [4]*Division of Pathology, Kobe University Graduate School of Medicine, Kobe,* [2]*Department of Pediatrics, Himeji Red Cross Hospital, Hyogo and* [3]*Center for Cell Therapy, Tokyo Medical and Dental University, Tokyo, Japan*

Key words ATG, CAEBV, cardiac muscle, Epstein-Barr virus, hematopoietic stem cell transplantation.

Chronic active Epstein–Barr virus infection (CAEBV) is a life-threatening disorder characterized by prolonged fever, wasting, hepatosplenomegaly, and cytopenia, in addition to abnormal EBV antibody titers and the presence of EBV antigens or EBV-DNA in tissue.[1] The ultimate prognosis of CAEBV is very poor, and patients with CAEBV often develop a progressive cellular and humoral immunodeficiency with pancytopenia and hypoglobulinemia that renders them susceptible to opportunistic infections or B- or T-cell lymphoproliferative disease.[2] An effective treatment for CAEBV has yet to be established, although allogeneic hematopoietic stem cell transplantation (HSCT) was recently reported to be effective in eradicating EBV-infected lymphocytes.[3,4] Allogeneic HSCT is, however, accompanied by a considerable risk of therapy-related death. Kimura *et al.* reported that approximately one-half of patients with CAEBV who underwent HSCT died within 60 days.[5]

We here report a patient with CAEBV who developed fatal acute circulatory failure during the preconditioning of HSCT, and in whom autopsy showed degeneration of specialized cardiac muscle and severe large-vessel arteritis associated with CAEBV.

Case report

A 12-year-old boy was admitted to a regional hospital with a 2 year history of fever, wasting, and a short stature (−3.0 SD). He was diagnosed with CAEBV on clinical signs and the presence of EBV genome in peripheral blood, and was admitted to hospital in April 2005. He was allergic to mosquito bites. On examination, lymph node adenopathy was noted. No skin lesion was observed. On brain CT, bilateral calcification of the basal ganglia was noted. Echocardiography showed dilatation of the lumens of the coronary arteries. The leukocyte count was 2.2×10^9/L; hemoglobin, 12.5 g/dL; and platelet count, 61×10^9/L. Biochemical analysis was as follows: aspartate aminotransferase, 28 IU/L; alanine aminotransferase, 14 IU/L; lactate dehydrogenase, 265 IU/L; C-reactive protein, 0.63 mg/dL; soluble interleukin-2 receptor, 1200 U/mL (normal, <519 U/mL); viral capsid antigen (VCA)-IgG, ×320; EA-IgG, ×10; EBNA, ×10. Natural killer activity was 55%. The quantity of EBV genome DNA was increased (2.6×10^3 copies/μg DNA) on polymerase chain reaction in peripheral blood mononuclear cell (PBMC), particularly in CD4+T cells (4.3×10^4 copies/μg DNA), but not in CD8+T cells or CD56+NK cells. Southern blot of the peripheral blood cells using an EBV terminal probe indicated monoclonal proliferation of EBV-infected lymphocytes. Flow cytometry showed the expression of the perforin protein in PBMC. Chromosome analysis of peripheral blood showed the normal male karyotype.

The high load of EBV genome DNA had persisted for over 6 months. Therefore, HSCT from a human leukocyte antigen (HLA)-matched sibling donor was planned in October 2005. Just before bone marrow transplantation, the quantity of EBV-DNA was consistently high in peripheral blood. No chemotherapy was performed prior to bone marrow transplantation. Echocardiography showed normal cardiac function. The exercise electrocardiogram (ECG) identified no abnormal findings.

The preparative conditioning regimen consisted of fludarabine (30 mg/m^2 × 4, day −7, −6, −5, −4), anti-thymocyte globulin (15 mg/kg × 5, day −7, −6, −5, −4, −3), and melphalan (70 mg/m^2 × 2, day −3, −2). Because anti-thymocyte globulin had been administered, he had suffered from high fever, skin rash, systemic arthralgia, and abdominal pain. He had wine-colored urine, but not microhematuria. He was diagnosed as having serum sickness and disseminated intravascular coagulation syndrome, and treated with gabexate mesilate, fresh frozen plasma, platelet transfusions, and methylprednisolone. Therefore, subsequent conditioning was all stopped on day −6 before transplantation. Blood examination showed elevation of liver enzymes, creatinine kinase, and coagulopathy. Fifty-two hours after the beginning of conditioning for transplant (on day −5), he suddenly developed acute circulatory failure. Just before cardiac arrest, echocardiogram showed normal cardiac function. Transient ventricular fibrillation was observed during resuscitation, but he soon developed cardiac arrest. Cardiac pulmonary resuscitation was unsuccessfully performed, and he died of sudden circulatory failure.

Correspondence: Daiichiro Hasegawa, MD, PhD, Department of Hematology and Oncology, Kobe Children's Hospital, 1-1-1, Suma, Kobe, Japan. Email: hasegawa_kch@hp.pref.hyogo.jp

Received 25 August 2007; revised 8 April 2008; accepted 9 April 2008.
doi: 10.1111/j.1442-200X.2009.02925.x

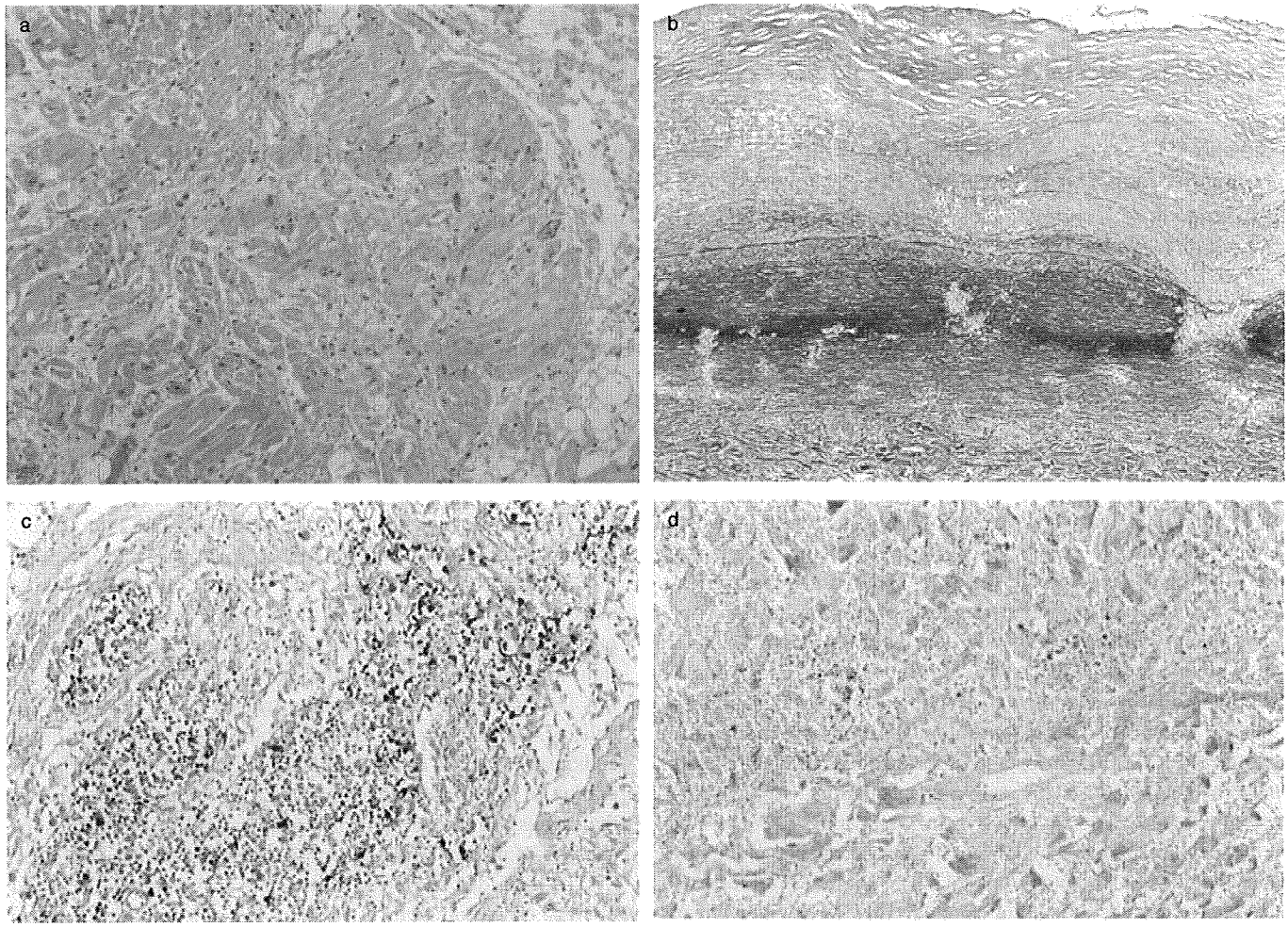

Fig. 1 (a) Histopathology showed degeneration of specialized cardiac muscle (HE), and mesoarteritis characterized by moth-eaten-appearing destruction of the medial elastic laminae, with (b) lymphocyte infiltration around the vasa vasorum and severe intimal thickening (EVG). (c) *In situ* hybridization demonstrated the presence of EBV-RNA (EBER-1) in the nuclei of lymphocytes in lymph nodes and (d) degenerated cardiac muscles.

At autopsy, histopathology showed not only EBV-associated hemophagocytic syndrome, but also degeneration of specialized cardiac muscle (Fig. 1a), mesoarteritis characterized by moth-eaten-appearing destruction of the medial elastic laminae (Fig. 1b), with $CD4^{+}$T lymphocyte infiltration around the vasa vasorum and severe intimal thickening. *In situ* hybridization demonstrated the presence of EBV-RNA (EBER-1) in the nuclei of lymphocytes in lymph nodes (Fig. 1c), around the vessels and degenerated cardiac muscles (Fig. 1d). Hemorrhagic tendency associated with disseminated intravascular coagulation syndrome was also noted at autopsy, although fatal intracranial hemorrhage and myocardial infarction were not documented. These findings suggested that hemophagocytic syndrome and degeneration of specialized cardiac muscles might have caused the fatal arrhythmia, considered to be associated with the EBV infection.

Discussion

In the current case, coronary artery dilatations and large-vessel arteritis were noted at autopsy. Arteritis has been reported in patients with CAEBV.[6] Coronary arteries and large vessels are often involved, and the presence of arteritis associated with EBV infection led to poor prognoses in those reported patients.[6–8] The vulnerability of systemic vessels caused by severe arteritis might be involved in the poor response to cardiopulmonary resuscitation. *In situ* hybridization showed that EBV-infected lymphocytes were associated with mesoarteritis, characterized by moth-eaten-appearing destruction of the medial elastic laminae and severe intimal thickening. Taken together, a comparatively high load of EBV-DNA persisting prior to transplantation might have been one of the factors promoting arteritis in this patient.

To our knowledge this is the first case reported in the literature in which the degeneration of specialized cardiac muscle was documented at autopsy in a patient with CAEBV. Transient ventricular fibrillation was also detected on ECG during cardiopulmonary resuscitation. The degeneration of specialized cardiac muscle might lead to fatal arrhythmia and acute circulatory failure. *In situ* hybridization also showed that EBER-positive

cells were scattered around cardiac muscle, suggesting that persistent EBV infection was involved in the degeneration of specialized cardiac muscle, Atrioventricular block has also been reported in EBV myocarditis.[9] These findings suggest that prolonged EBV infection could lead to the degeneration of specialized cardiac muscle over a long period.

Allogeneic HSCT has been reported to offer a good prognosis for those with CAEBV.[2,3] A national survey, however, performed in Japan found a high risk of HSCT-related mortality.[5] Recently, successful treatment of CAEBV infection using reduced-intensity stem cell transplantation (RIST) with fludarabine and melphalan with or without anti-thymocyte globulin (ATG) has been reported,[10,11] which could control CAEBV by reconstituting host immunity against EBV. Regimen-related toxicity is expected to be more effectively alleviated using a reduced intensity conditioning regimen for transplantation than by conventional myeloablative conditioning in those with an impaired residual function of organs. In contrast, it is well known that the risk of EBV-related complications after transplantation might increase with the additional use of ATG in non-myeloablative conditioning.[12] In the current case we adapted fludarabine-based, reduced-intensity conditioning with ATG for transplantation, because it was predicted that the residual cardiac function might be partly impaired by coronary giant aneurysms. The additional use of ATG, however, in fludarabine-based conditioning might have been involved in the development of hemophagocytic syndrome during preconditioning for transplantation, although it was uncertain whether the serum sickness induced by the use of ATG was associated with the degeneration of cardiac muscle. To answer the many remaining questions, such as the best transplantation method for CAEBV, the optimal timing of transplantation, and the most effective conditioning regimen, a multicenter-based clinical trial is needed.

References

1 Cohen JI. Epstein-Barr virus infection. *N. Engl. J. Med.* 2000; **343**: 481–92.
2 Okano M. Overview and problematic standpoints of severe chronic active Epstein-Barr virus infection syndrome. *Crit. Rev. Oncol. Hematol.* 2002; **44**: 273–82.
3 Fujii N, Takenaka K, Hiraki A *et al.* Allogeneic peripheral blood stem cell transplantation for the treatment of chronic active Epstein-Barr virus infection. *Bone Marrow Transplant.* 2000; **26**: 805–8.
4 Okamura T, Hatsukawa Y, Arai H, Inoue M, Kawa K. Blood stem-cell transplantation for chronic active Epstein-Barr virus with lymphoproliferation. *Lancet* 2000; **356**: 223–4.
5 Kimura H, Hoshino Y, Kanegane H *et al.* Clinical and virologic characteristics of chronic active Epstein-Barr virus infection. *Blood* 2001; **98**: 280–86.
6 Murakami K, Ohsawa M, Hu SX, Kanno H, Aozasa K, Nose M. Large-vessel arteritis associated with chronic active Epstein-Barr virus infection. *Arthritis Rheum.* 1998; **41**: 369–73.
7 Kikuta H, Sakiyama Y, Matsumoto S *et al.* Detection of Epstein-Barr virus DNA in cardiac and aortic tissues from chronic, active Epstein-Barr virus infection associated with Kawasaki disease-like coronary artery aneurysms. *J. Pediatr.* 1993; **123**: 90–92.
8 Kimura H, Morishima T, Kanegane H *et al.* Prognostic factors for chronic active Epstein-Barr virus infection. *J. Infect. Dis.* 2003; **187**: 527–33.
9 Reitman MJ, Zirin HJ, DeAngelis CJ. Complete heart block in Epstein-Barr myocarditis. *Pediatrics* 1978; **62**: 847–9.
10 Sakata N, Sato E, Sawada A, Yasui M, Inoue M, Kawa K. Chronic active Epstein-Barr virus infection treated with reduced intensity stem cell transplantation. *Rinsho Ketsueki* 2004; **45**: 393–6.
11 Sawada A, Inoue M, Koyama M *et al.* Complete elimination of endogenous EBV after allogeneic UCBT. *Rinsho Ketsueki* 2007; **48**: 1111.
12 Brunstein CG, Weisdorf DJ, DeFor T *et al.* Marked increased risk of Epstein-Barr virus-related complications with the addition of antithymocyte globulin to a nonmyeloablative conditioning prior to unrelated umbilical cord blood transplantation. *Blood* 2006; **108**: 2874–80.

Congenital upper thoracic spondyloptosis with multiple other associated anomalies

Banu Alicioglu, Mustafa Kemal Demir and Yavuz Durmus
Radiology Department, Trakya University School of Medicine, Edirne, Turkey

Key words congenital abnormalities, diagnosis, scapula, spine, spondyloptosis.

Spondyloptosis indicates any slip greater than 100% of a vertebral body on another or an extreme degree of spondylolisthesis. It is rare and there are isolated case reports in the literature.[1,2] It frequently involves the L5–S1 level. It is attributed to congenital dysplasia of the articular process.[3]

Correspondence: Banu Alicioglu, MD, School of Medicine, Trakya University, 22030 Edirne, Turkey. Email: banualicioglu@trakya.edu.tr

Received 4 September 2007; revised 8 February 2008; accept 18 March 2008.
doi: 10.1111/j.1442-200X.2009.02926.x

Presented here is the case of an infant with severe spinal cord compression related to T2–3 spondyloptosis and multisegmental vertebral congenital anomaly. Multisystem anomalies such as Sprengle deformity, situs inversus totalis, and right renal agenesis were also demonstrated radiologically. To our knowledge this is a unique case of thoracic spondyloptosis.

Case report

A 9-month-old male infant presented with a significant delay in head holding. He was the result of the first pregnancy of young