Long-Term Parvovirus B19 Infections With Genetic Drift After Cord Blood Transplantation Complicated by Persistent CD4+ Lymphocytopenia

Michio Suzuki, MD, Yoshinori Ito, MD, PhD,* Akira Shimada, MD, PhD,* Mika Saito, PhD,† Hideki Muramatsu, MD, PhD,* Asahito Hama, MD, PhD,* Yoshiyuki Takahashi, MD, PhD,* Hirokazu Kimura, PhD,‡ and Seiji Kojima, MD, PhD**

Summary: A 5-month-old girl was diagnosed with Langerhans cell histiocytosis and received unrelated umbilical cord blood transplantation at the age of 14 months. After cord blood transplantation, CD4+ lymphocytopenia from unknown causes was observed, and persistent infections with human parvovirus B19 (B19) occurred. We performed repeated longitudinal genetic analysis for B19, which revealed 6 nucleotide mutations in B19 nonstructural protein regions in the patient. The resulting changes of the nonstructural 1 structure may have altered antigenicity of the virus and could play a role in the pathogenesis of persistent infection under immunocompromised conditions.

Key Words: cord blood transplantation, genetic drift, lymphocytopenia, parvovirus B19

(*J Pediatr Hematol Oncol* 2014;36:e65–e68)

Human parvovirus B19 (B19) is a nonenveloped, 5.5 kb, single-strand DNA virus. The virus has 2 major open reading frames, one encoding the nonstructural protein, nonstructural (NS) 1, and the other encoding the capsid proteins, viral protein (VP) 1 and VP2.[1] Three B19 genotypes (1, 2, and 3) have been described. Genotypes 2 and 3 are genetically distant from genotype 1 with nucleotide divergence of >11%.[1] Genotypes 2 and 3 are infrequently detected in clinical specimens, but the frequency was shown to be much higher in 1 prospective study. The true prevalence and clinical relevance of these differences are not fully known. In addition to causing erythema infectiosum, the B19 virus also causes arthropathy in adults, particularly in middle-aged women, aplastic crisis in patients with hemolytic anemia, chronic hypoplastic anemia in immunocompromised hosts, and fetal hydrops as a result of infection during pregnancy.[2] It has also been associated with autoimmune diseases including rheumatoid arthritis and systemic lupus erythematosus.[3] In general, infection is transient in immunocompetent patients; however, persistent infection has been reported in immunocompromised patients such as those with human immunodeficiency virus (HIV).[4] The B19 virus is a rare cause of posttransplant viral infection,[5] and there have been a few reports of persistent infections after stem cell transplantation.[6]

We report a case of persistent infections with B19 virus in a patient who developed persistent CD4+ lymphocytopenia after cord blood transplantation (CBT) for Langerhans cell histiocytosis. We studied longitudinal changes in viral DNA load and genetic changes of B19 during the course of these persistent infections.

MATERIALS AND METHODS

EDTA-treated peripheral blood was prospectively collected after transplantation for routine blood examinations and to monitor for Epstein-Barr virus, cytomegalovirus, and human herpesvirus 6 DNA until discharge. After discharge, blood samples were obtained at each patient visit for routine blood examinations. When the patient showed hemolytic anemia or after B19 DNA became positive in whole blood samples, B19 DNA was also measured. A biopsy specimen from a circumanal ulcer was also obtained when Crohn disease was suspected. DNA was extracted from 200 μL of whole blood and biopsy tissue using QIAamp blood kits (Qiagen, Hilden, Germany) and TaKaRa DEXPAT (Takara, Ohtsu, Shiga, Japan), respectively. Real-time quantitative polymerase chain reaction assay for B19 was performed as described previously.[7,8]

For genetic analysis of B19, 2 B19 viremic samples at different time points of persistent B19 infection were analyzed by DNA sequencing as described previously.[9] The primers were used for sequencing the NS1/VP1/VP2 regions. Then, B19 sequences were aligned using Clustal W (http://clustalw.ddbj.nig.ac.jp/top-j.html) for genotypic analysis. Genetic distances were calculated using the Kimura 2-parameter method,[10] and phylogenetic trees were constructed by the neighbor-joining method.[11] Results of phylogenetic trees were visualized using TreeExplorer software version 2.12 (http://www.ctu.edu.vn/~dvxe/Bioinformatic/Software/BIT%20Software/TE_man.html). Prototype B19 sequences from GenBank were used as reference sequences (accession numbers were as follows: genotype 1, M24682 [B19-Wi], M13178 [B19-Au]; genotype 2, AY064475 [B19-A6], AY044266 [B19-Wi]; genotype 3, AX003421 [B19-V9], AY083234 [B19-D91.1]).

The protocol of this study was approved by the Nagoya University Institutional Review Board, and written informed consent was obtained from the parents of the patient.

CASE REPORT

A 5-month-old girl was diagnosed with multisystem Langerhans cell histiocytosis. Although she received 2 courses of DAL

Received for publication August 22, 2012; accepted August 20, 2013.
From the *Department of Pediatrics, Nagoya University Graduate School of Medicine, Nagoya; †Gunma Prefectural Institute of Public Health and Environmental Sciences, Gunma; and ‡Infectious Disease Surveillance Center, National Institute of Infectious Diseases, Tokyo, Japan.
The authors declare no conflict of interest.
Reprints: Seiji Kojima, MD, PhD, Department of Pediatrics, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan (e-mail: kojimas@med.nagoya-u.ac.jp).

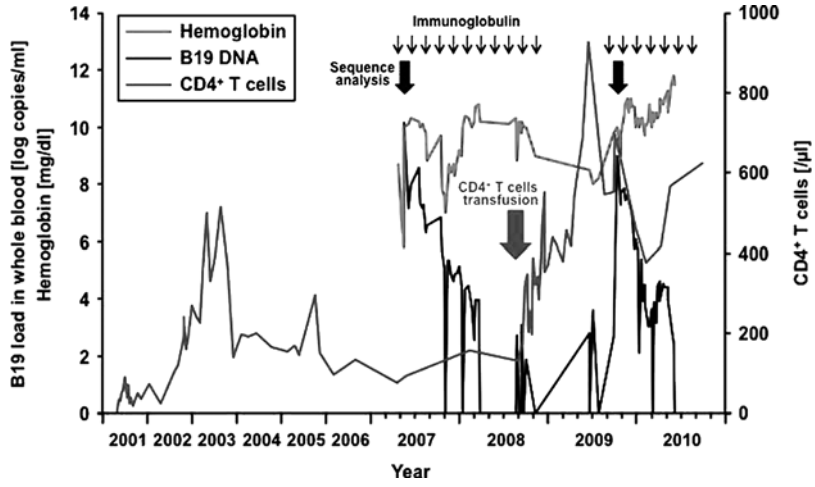

FIGURE 1. Time course of the number of CD4+ T cells and parvovirus B19 viral load in whole blood in relation to representative laboratory data after cord blood cell transplantation. Levels of viral DNA in whole blood samples were measured by real-time quantitative polymerase chain reaction assays. Genetic analyses at the first and the second peak of viremia (black large arrows) were performed. Short arrows indicate weekly intravenous immunoglobulin treatment at 400 mg/kg/d. Blue arrow represents the time of CD4+ T-cell transfusion.

HX-83 induction therapy, which includes prednisone, vinblastine, and etoposide, her disease did not improve. She received unrelated umbilical CBT at 14 months of age (April 2001). The conditioning regimen consisted of cyclophosphamide (60 mg/kg) on days −4 and −3; antithymocyte globulin (2.5 mg/kg) on days −5, −4, −3, and −2; and total body irradiation (2.5 Gy) twice a day on days −2 and −1. Methotrexate and tacrolimus were given for prophylaxis against graft-versus-host disease (GVHD). Neutrophil engraftment was documented on day 24 and full donor chimerism was observed after CBT; however, the number of CD4+ T cells did not go above 100/μL for 1 year after transplantation and CD4+ lymphocytopenia persisted for >7 years (Fig. 1). Her IgG, IgM, and IgA were 835, 42, and 32 mg/dL, respectively, at day 35 after transplantation. Stimulation tests with phytohemagglutinin and concanavalin A showed low responses. Natural killer cell activity was in the normal range (24%) at day 75 after transplantation. She suffered from grade 1 acute GVHD, but chronic GVHD did not develop.

She developed cough and fever at the age of 7 (May 2007). Her hemoglobin level was 5.8 g/dL and reticulocytes showed 0.4%. A bone marrow aspiration showed hypoplastic marrow with poor erythroid elements. Specific IgM antibodies for B19 were positive. B19 DNA in a whole blood sample was 1.4×10^{10} copies/mL. Underlying causes, such as thymoma, secondary cancer, drugs, and autoimmune disorders, were excluded. She received a 3-day course of intravenous immunoglobulin (400 mg/kg) daily for treatment of pure red cell aplasia. B19 viremia persisted, and the reticulocyte count did not improve, so she was treated repeatedly with 400 mg/kg/d immunoglobulin every week; she did not receive any blood transfusions. In September 2008, she was transfused with activated CD4+ T lymphocytes (3.4×10^7/kg) that were prepared from her own mononuclear cells as described previously.[12] Her CD4+ cells were over 500/μL after infusion (Fig. 1). Viremia improved slowly after weekly immunoglobulin treatment started, and this treatment was continued until January 2009 (Fig. 1). In October 2009, her reticulocyte count and hemoglobin level were 0.5% and 9 g/dL, respectively, and her level of B19 DNA was 9.8×10^8 copies/mL. Weekly immunoglobulin treatment was restarted.

She had a fever and noticed multiple oral aphtha, which became increasingly severe in January 2010. Crohn disease was suspected due to a circumanal ulcer and a red papule on the dorsal surface of the left hand, which occurred in February 2010. Histopathologic examination of biopsy samples from the ulcer showed nonspecific inflammation and B19 DNA was detected (1.49×10^5 copy/μg) in the biopsy specimen. Oral aphtha, the circumanal ulcer, and fever improved in April 2010 without any specific treatment besides weekly immunoglobulin treatment. B19 DNA disappeared in June 2010.

RESULTS AND DISCUSSION

Cytomegalovirus, Epstein-Barr virus, and herpes simplex virus are common causes of posttransplantation viral infection, whereas infection with B19 virus is rare after hematopoietic stem cell transplantation. The incidence of primary or recurrent infection with B19 in patients after allogenic hematopoietic stem cell transplantation seems to be 1% to 2%.[13] Eid et al[5] investigated 98 cases of posttransplantation B19 infection, including 24 cases after hematopoietic stem cell transplantation. Anemia was observed in 98.8% of patients with B19 infection. Fever and flu-like manifestations occurred in 25.9% of patients and skin rash occurred in 13.3% of patients. B19 infection occurred between 1 week and 96 months after transplantation, with a

TABLE 1. Nucleotide Substitutions Found in Longitudinal Isolates

	Nucleotide					
	941	**1037**	**1048**	**1112**	**1118**	**1266**
Date						
May 24, 2007	T/C	T/C	A	T	T	A
October 19, 2009	T	T	A/G	C	T/C	A/G
Amino acid change			K→K/R			T→T/A

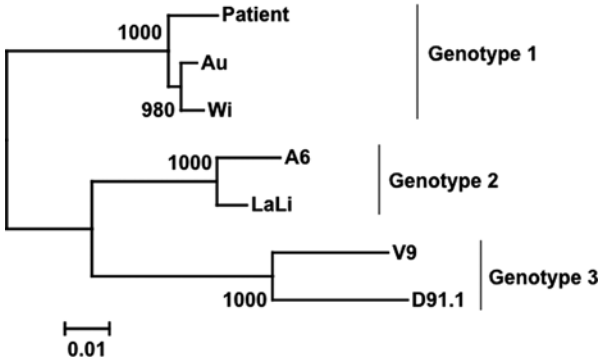

FIGURE 2. Phylogenetic tree constructed from VP1 sequence. The HPV B19 sequence from the patient was aligned with reference sequences by GenBank (genotype 1, B19-Wi and B19-Au; genotype 2, B19-A6 and B19-Wi; genotype 3, B-19-V9 and B19-D91.1). Genetic distances were calculated using the Kimura 2-parameter method and phylogenetic trees were constructed using the neighbor-joining method.

median onset time of 1.8 months. Recurrence of B19 infection occurred in 23% of cases. In the present case, onset occurred 76 months after CBT, causing symptomatic persistent infection for >3 years. Repeated administration of immunoglobulin was effective and B19 DNA disappeared. However, anemia and B19 viremia may recur, because $CD4^+$ lymphocytopenia has not been cured.

B19 infection is known to cause persistent pure red-cell aplasia in immunocompromised patients in whom B19 virus-specific antibody production is insufficient.[2,14] Previous reports have described persistent infection with B19 in patients with congenital immunodeficiency syndrome,[2] patients receiving cytotoxic chemotherapy or immunosuppressive drugs,[2] patients undergoing bone marrow transplantation[5,6] or organ transplantation,[5] and patients with HIV infection.[4] In the present case, persistent $CD4^+$ lymphocytopenia of unknown cause was present after transplantation, which likely caused the subsequent persistent B19 infection. CD4 deficiency has been shown to be present in HIV infection and idiopathic $CD4^+$ lymphocytopenia, in which opportunistic infections can occur. Cryptococcus is one of the more common infections in patients with CD4 penia.[15] Zonios et al[16] reported that cryptococcal and nontuberculous mycobacterial infections were the major presenting opportunistic infections of idiopathic $CD4^+$ lymphocytopenia in 33.3% (13/39) and 20.5% (8/39) of cases, respectively. Infections caused by cytomegalovirus, Epstein-Barr virus, human herpesvirus 8, human papillomavirus, JC virus, and varicella zoster virus were reported in a small number of cases.[16] The present case suffered from long-standing infections with B19, and this pathogen is rare in a patient with idiopathic $CD4^+$ lymphocytopenia.

To our knowledge, this is the first report of idiopathic $CD4^+$ lymphocytopenia after hematopoietic stem cell transplantation. The treatment of idiopathic $CD4^+$ lymphocytopenia mainly consists of prophylaxis and treatment of opportunistic infections and attempts to increase $CD4^+$ T-cell counts. Interleukin-2 therapy is an option to increase $CD4^+$ counts along with other cytokines such as interferon-γ and interleukin-7.[15] Hematopoietic stem cell transplantation may be another treatment option to restore $CD4^+$ T-cell count and immune function in idiopathic $CD4^+$ lymphocytopenia.[17] The present case is an unfavorable example of hematopoietic stem cell transplantation as a cause.

To characterize B19 in this patient, sequence analysis of the coding regions of 3 major B19 proteins, that is, NS1, VP1, and VP2, in blood samples was performed. Analyses at the first and the second peak of viremia (Fig. 1) revealed sequence variations at 6 positions in the NS1 area; 2 of them were nonsynonymous mutations, which altered the amino acid sequence of the protein (Table 1). These changes may have influenced the protein structure and biological function of NS1, thus affecting the course of infection. NS1 protein is known to be cytotoxic and also autoregulates its own transcription, which may affect synthesis of NS1 protein.[18] In addition, changes in the NS1 protein structure may alter antigenicity and the recognition of humoral antibodies that confer resistance to immunoglobulin infusions. This patient received intravenous immunoglobulin for treatment of B19 infection, and these genetic changes may have been caused by selection of resistant clones, although direct evidence of this selection was not shown in the present study. Further studies are needed to clarify the effects that arise from amino acid changes. Although B19 is usually stable genetically, and mutations seldom occur during the course of infection, genetic drift of B19 in acquired immunodeficiency syndrome patients with CD4 lymphocytopenia has been reported.[1]

Phylogenic analysis on the basis of the VP1 region, which was identical between the 2 different samples, revealed that this B19 virus was identified as genotype 1 (Fig. 2), which usually causes acute and transient infection.[19] Servant et al[19] reported that most genotype 2 and 3 viruses were isolated in patients older than those infected with genotype 1 virus, frequently in association with immunodeficiency. They hypothesized that adult patients with impaired immunity, who are likely to have experienced a previous genotype 1 virus infection, might be reinfected by a genotype 2 or 3 virus. However, the current patient had no blood transfusions and had been kept in the hospital, suggesting that superinfection of another B19 was not likely. B19 infections are ubiquitous and can cause an asymptomatic infection including a mild respiratory tract illness with no rash. The index case is generally unrecognized in nosocomial infections.[20] Therefore, identification of the source of B19 infection is usually difficult.

In this case report, idiopathic $CD4^+$ lymphocytopenia was unexpectedly observed after CBT, and infections with B19 persisted for several years. The resulting changes of the NS1 during persist infection may have altered antigenicity of the virus and could have played a role in the pathogenesis of persistent B19 infection under $CD4^+$ lymphocytopenia.

REFERENCES

1. Hung CC, Sheng WH, Lee KL, et al. Genetic drift of parvovirus B19 is found in AIDS patients with persistent B19 infection. *J Med Virol*. 2006;78:1374–1384.
2. Young NS, Brown KE. Parvovirus B19. *N Engl J Med*. 2004;350:586–597.
3. Lunardi C, Tinazzi E, Bason C, et al. Human parvovirus B19 infection and autoimmunity. *Autoimmun Rev*. 2008;8:116–120.
4. Frickhofen N, Abkowitz JL, Safford M, et al. Persistent B19 parvovirus infection in patients infected with human

immunodeficiency virus type 1 (HIV-1): a treatable cause of anemia in AIDS. *Ann Intern Med*. 1990;113:926–933.
5. Eid AJ, Brown RA, Patel R, et al. Parvovirus B19 infection after transplantation: a review of 98 cases. *Clin Infect Dis*. 2006;43:40–48.
6. Plentz A, Hahn J, Holler E, et al. Long-term parvovirus B19 viraemia associated with pure red cell aplasia after allogeneic bone marrow transplantation. *J Clin Virol*. 2004; 31:16–19.
7. Shibata Y, Kitajima N, Kawada J, et al. Association of cytomegalovirus with infantile hepatitis. *Microbiol Immunol*. 2005;49:771–777.
8. Wada K, Kubota N, Ito Y, et al. Simultaneous quantification of Epstein-Barr virus, cytomegalovirus, and human herpesvirus 6 DNA in samples from transplant recipients by multiplex real-time PCR assay. *J Clin Microbiol*. 2007;45:1426–1432.
9. Toan NL, Duechting A, Kremsner PG, et al. Phylogenetic analysis of human parvovirus B19, indicating two subgroups of genotype 1 in Vietnamese patients. *J Gen Virol*. 2006;87: 2941–2949.
10. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol*. 1980;16:111–120.
11. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol*. 1987; 4:406–425.
12. Tomizawa D, Aoki Y, Nagasawa M, et al. Novel adopted immunotherapy for mixed chimerism after unrelated cord blood transplantation in Omenn syndrome. *Eur J Haematol*. 2005;75:441–444.
13. Hayes-Lattin B, Seipel TJ, Gatter K, et al. Pure red cell aplasia associated with parvovirus B19 infection occurring late after allogeneic bone marrow transplantation. *Am J Hematol*. 2004;75:142–145.
14. Anderson MJ, Higgins PG, Davis LR, et al. Experimental parvoviral infection in humans. *J Infect Dis*. 1985;152:257–265.
15. Luo L, Li T. Idiopathic $CD4^+$ lymphocytopenia and oppotunistic infection - an update. *FEMS Immunol Med Microbiol*. 2008;54:283–289.
16. Zonios DI, Falloon J, Bennett JE, et al. Idiopathic $CD4^+$ lymphocytopenia: natural history and prognostic factors. *Blood*. 2008;112:287–294.
17. Petersen EJ, Rozenberg-Arska M, Dekker AW, et al. Allogenic bone marrow transplantation can restore $CD4^+$ T-lymphocyte count and immune function in idiopathic $CD4^+$ T-lymphocytopenia. *Bone Marrow Transplant*. 1996;18:813–815.
18. Hemauer A, von Poblotzki A, Gigler A, et al. Sequence variability among different parvovirus B19 isolates. *J Gen Virol*. 1996;77:1781–1785.
19. Servant A, Laperche S, Lallemand F, et al. Genetic diversity within human erythroviruses: identification of three genotypes. *J Virol*. 2002;76:9124–9134.
20. Cherry DJ, Schulte DJ. Human parvovirus B19. In: Feigin RD, Cherry JD, Demmler-Harrison GJ, Kaplan SL, eds. *Feigin and Cherry's Textbook of Pediatric Infectious Diseases*. 6th ed. Philadelphia: Saunders; 2009:1902–1920.

