Persistent symptomatic parvovirus B19 infection with severe thrombocytopenia transmitted by red blood cell transfusion containing low parvovirus B19 DNA levels

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BACKGROUND: Transfusion-mediated human parvovirus B19 (PVB19) infection is rare but often causes severe hematologic disorders. In Japan, routine blood donor screening for PVB19 antigen (detection sensitivity, $10^{6.4}$ IU/mL) using a chemiluminescent enzyme immunoassay (CLEIA) was introduced in 2008. However, there is no consensus on the minimal infectious dose of PVB19 permissible for red blood cells (RBCs).

CASE REPORT: A 64-year-old man, who had received hemodialysis for diabetic nephropathy for 5 years, underwent an RBC transfusion for anemia caused by hemorrhagic enterocolitis. He developed persistent high fever and progressive thrombocytopenia. He was diagnosed with PVB19 infection when a marrow examination showed giant erythroblasts, and his serum was positive for PVB19 DNA. His serum was negative for PVB19 immunoglobulin (Ig)M and IgG before transfusion, but positive for both after transfusion. This PVB19 infection was deemed to be transmitted by the RBC transfusion because low levels of PVB19 DNA (1.10×10^4 IU/mL) were detected in one of the blood donors. A DNA homology test of PVB19 showed complete genomic identity between the virus in the donor and our patient.

CONCLUSION: We report a patient who developed persistent PVB19 infection from an RBC transfusion containing low levels of PVB19. This is the second case of transfusion-mediated PVB19 infection since the introduction of CLEIA in 2008. Transmission may occur in immunocompromised patients lacking PVB19-neutralizing antibodies. The report of further such cases will allow the establishment of minimal threshold values and more effective screening tests for PBV19 transmission through RBC products.

Human parvovirus B19 (PVB19) is a single-stranded DNA virus discovered in 1975.[1] This ubiquitous virus usually causes erythema infectiosum in children, but can also affect adults. Adult infections with PVB19 are mainly transmitted from pediatric patients through the respiratory route, and transmission through transfusion is rare. Various reports have shown that plasma products with low viral loads ($<10^4$ IU/mL) are safe for recipients.[2,3] However, there has been no consensus on the threshold values for PVB19 transmission in red blood cell (RBC) products or in immunocompromised patients. This infection usually resolves spontaneously within 2 weeks and requires no medication,[4] except in patients with hemolytic anemia. However, persistent infection has been reported, especially in immunocompromised hosts.[5] We report a patient who developed persistent thrombocytopenia and concomitant PVB19 infection transmitted through the transfusion of RBCs with a low viral load (1.10×10^4 IU/mL).

ABBREVIATIONS: CLEIA = chemiluminescent enzyme immunoassay; GP = glycoprotein; PVB19 = human parvovirus B19.

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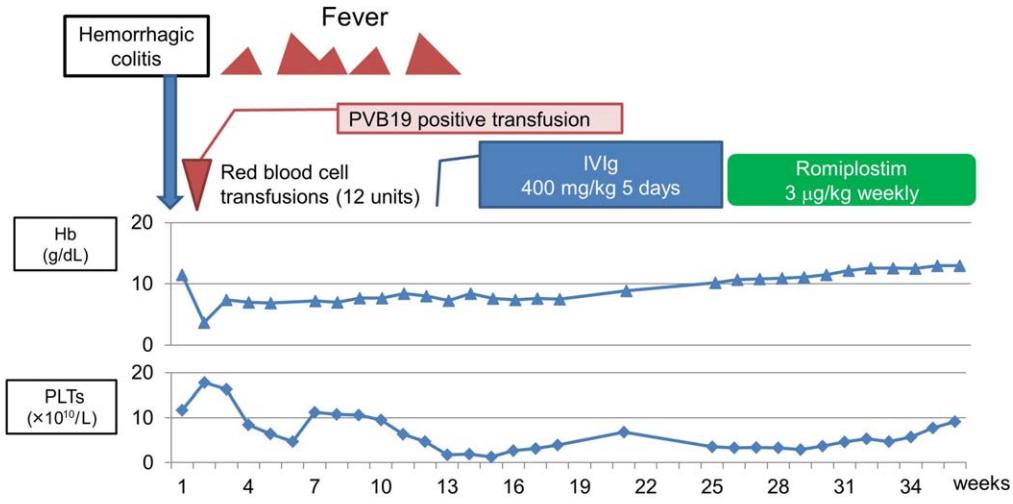

	Pre-transfusion	Two months after transfusion	Four months after transfusion
PVB19 DNA (serum)*	Negative	Positive	Positive
PVB19 DNA (bone marrow)*	Not examined	Positive	Positive
PVB19 IgM (serum)*	0.05	Not examined	6.49
PVB19 IgG (serum)*	0.29	Not examined	9.72

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Fig. 1. Clinical course. [Color figure can be viewed at wileyonlinelibrary.com]

CASE REPORT

A 64-year-old man was admitted to our hospital with fever of unknown origin and progressive thrombocytopenia. His past medical history included insulin-requiring Type 2 diabetes mellitus, hypertension, and end-stage renal failure caused by diabetic nephropathy, with dialysis for more than 5 years.

One month before his admission, he developed bloody diarrhea. He was diagnosed with hemorrhagic bacterial enterocolitis and received 12 units of RBCs (1 unit included 140 mL of RBCs) and antibiotic infusions. His symptoms resolved in 2 weeks. He then developed fever and thrombocytopenia and was referred to a hematologist. His clinical course is shown in Fig. 1.

Laboratory data revealed thrombocytopenia, normocytic anemia, and elevated C-reactive protein. A marrow examination revealed hyperplastic marrow without malignancy. The myeloid-to-erythroid precursor cell ratio (100:1) indicated extensive erythroid hypoplasia. A few giant erythroblasts were observed (Figs. 2A, 2C, and 2E). Computed tomography and 67Ga-citrate scintigraphy showed no inflammatory sites.

The patient was diagnosed with PVB19 infection based on his clinical presentation and the detection of PVB19 DNA in his peripheral blood. The transmission of PVB19 by RBC transfusion was suspected for the following reasons: 1) the patient had been in hospital during the incubation period (usually 4-15 days); 2) the archived serum sample of one of the blood donors was positive for both anti-PVB19 immunoglobulin (Ig)M and IgG on enzyme-linked immunosorbent assays (ELISAs; anti-PVB19 IgM, ELISA S/CO ratio 4.36; anti-PVB19 IgG, ELISA S/CO ratio 12.3); and 3) a low titer (1.10×10^4 IU/mL) of PVB19 DNA was detected in the sample. PVB19 DNA was amplified and quantified with the *artus* Parvo B19 TM polymerase chain reaction kit (Qiagen KK). The DNA sequence in the NS1/VP1 region of PVB19 (1069 bp) was analyzed, according to a previous report.[6] This revealed the complete identity of the viral sequences in the patient and donor, confirming the transmission of PVB19 through RBC products (all sequences are shown in Fig. S1, available as supporting information in the online version of this paper).

Over the next 2 months, the patient's fever continued and he developed pancytopenia. A second marrow aspiration 4 months after transfusion showed hypocellular marrow with no giant erythroblasts (Figs. 2B and 2D). Both the patient's serum and marrow remained positive for PVB19 DNA, and persistent PVB19 infection was suspected. Although his posttransfusion corrected platelet (PLT) count increment was 10.5×10^9/L, which was not significantly low, coexisting immune thrombocytopenic purpura could not be excluded because anti-glycoprotein (GP) antibodies for GPIIb/IIIa, GPIb/IX, GPIV, and HLA Class I were detected in his serum.

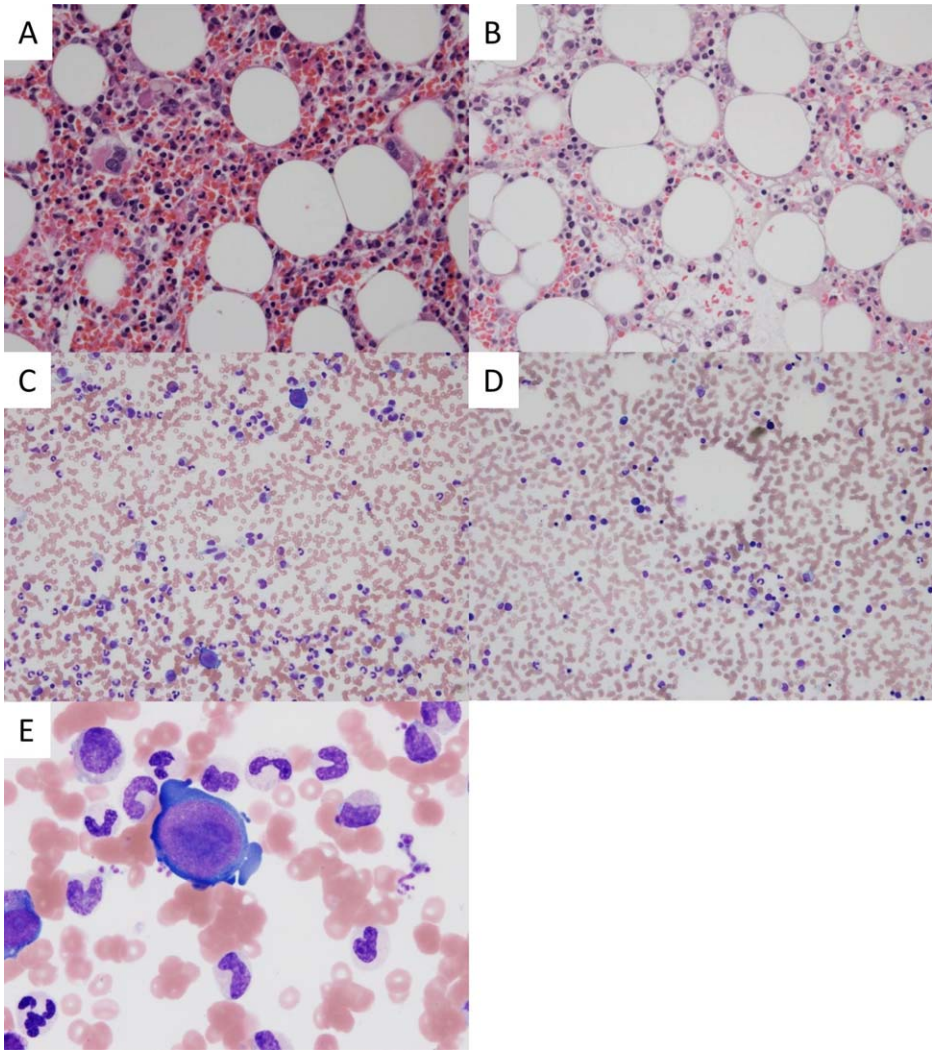

Fig. 2. Histopathologic findings in the bone marrow. (A, B) Hematoxylin-eosin–stained clot sections at diagnosis (2 months after transfusion; A, 400×) and 4 months after transfusion (B, 400×). Marrow analysis at diagnosis showed hypercellular marrow with a predominance of granulocytes (A). Evaluation at 4 months after transfusion revealed hypocellular marrow (B). (C-E) May-Grunwald-Giemsa staining of a marrow smear at diagnosis (2 months after transfusion; C, 400×; E, 1000×) and at 4 months after transfusion (D, 400×). Giant erythroblasts were seen at diagnosis (C, E), but were not detected 4 months after transfusion (D). [Color figure can be viewed at wileyonlinelibrary.com]

Intravenous immunoglobulins (IVIGs; 400 mg/day) were administered for 5 days for both his persistent PVB19 infection and his immune thrombocytopenic purpura. Shortly thereafter, his fever resolved. Three weeks after the initiation of IVIG therapy, the patient's PLT count started to increase, but remained below 5.0×10^{10}/L. After the administration of 3 μg/kg romiplostim, a thrombopoietin agonist, the patient required no further PLT transfusion (Fig. 1). Although his pretransfusion serum was negative for both anti-PVB19 IgM and IgG, it was positive for both immunoglobulins 4 months after transfusion. These data confirm that the patient had suffered a recent PVB19 infection. His serum PVB19 DNA remained positive 2 and 4 months later.

DISCUSSION

This case demonstrates that persistent PVB19 infection can be transmitted through the transfusion of RBCs containing a low titer of PVB19 DNA. In general, serum contamination is much lower in RBC products than in the other products. The threshold value for the transmission of PVB19 in RBC products is less clear than that for its transmission in plasma-derived products. Based on studies of PVB19 transmission through solvent- or detergent-treated pooled plasma,[2,7] the US Food and Drug Administration recommends that the viral load of PVB19 DNA in the plasma pools used for the manufacture of plasma-derived products should not exceed 10^4 IU/mL, to reduce

the potential risk of transmission through these products. In the United States, only plasma-derived products are screened for PVB19. It has been shown that the serum remains positive for PVB19 DNA for a long time, even in healthy patients.[8] The minimal threshold level of PVB19 in cellular products required for the transmission of the virus remains undefined, and there is no consensus on the value for single-donor components, especially RBCs. Kleinman and colleagues[9] and Yu and colleagues[10] suggested that blood cell products containing less than 10^6 IU/mL PVB19 DNA are not infectious. In Germany, Schmidt and colleagues[11] showed that blood products with less than 10^5 IU/mL PVB19 DNA were not infectious because neutralizing antibodies were also present in the RBC products. Nonetheless, our case shows that the transmission of PVB19 can occur from a donor with low levels of PVB19 DNA (1.10×10^4 IU/mL) who is anti-PVB19 IgM and IgG positive. In this study, a low titer (1.10×10^4 IU/mL) of PVB19 DNA was detected in one donor's archived serum sample. Although the parvovirus DNA titer in the RBCs was unknown, a normal RBC product (240 mL) usually contains approximately 20 mL of the donor's plasma.

In Japan, PVB19 infection through transfusion has been reported in only 10 cases (six patients with malignancy, two patients with hemolytic anemia, and two pregnant women) within the past 15 years.[12,13] Since 2008, all Japanese donors have been checked for PVB19 antigen with a chemiluminescent enzyme immunoassay (CLEIA) using the parvovirus B19 detection kit on an automated CLEIA analyzer (CL4800, Fujirebio Diagnostics, Inc.). The detection limit of the CLEIA has been reported to be equivalent to $10^{6.4}$ IU/mL.[13] A previous report showed a correlation between PVB19 antigen and the PVB19 DNA load, using the source material of the World Health Organization PVB19 genotype panels (M1S, M2S, and M3S).[14] After the introduction of the CLEIA in 2008, only one pregnant woman was reported to be infected with PVB19 transmitted through RBC products, but the PVB19 DNA level in her donor was unknown. Therefore, this is the first report in Japan since 2008 of a PVB19 infection transmitted by RBC transfusion in which the PVB19 DNA in the donor was quantified.

Sakata and coworkers[15] showed that the viral load of PVB19 did not exceed 10^4 IU/mL in any of the plasma pools from donors who were negative for PVB19 according to CLEIA. The viral load of PVB19 in the RBC product transfused into our patient appeared to be around the lower limit of the CLEIA screening test. In our patient, the virus may have been transmitted from a blood product containing such low levels of PVB19 DNA because he was immunocompromised, suffering from diabetic nephropathy with dialysis, and had no PVB19-neutralizing antibodies before transfusion. Nonetheless, blood transfusion is often required by immunocompromised patients. Furthermore, recent immunomodulatory therapies, such as rituximab, are reported to induce a higher incidence of viral infection.[16,17] Therefore, a more effective screening test for PVB19 is required. Donated blood is currently universally screened for PVB19 only in Poland, Germany, Austria, and Japan.[18] Such screening should be undertaken in more countries.

In conclusion, we have reported a patient suffering PVB19 infection with severe thrombocytopenia, transmitted by an RBC transfusion containing low levels of PVB19 DNA. The transmission of PVB19 may be attributable to the recipient's immunologic status and lack of PVB19-neutralizing antibodies. A more effective screening method is required to prevent the transmission of PVB19 through RBC products.

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CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Fig. S1. Comparison of the PVB19 DNA sequences in the patient and donor. Nested PCR was performed with both the patient's and donor's sera using methods described previously.[6] Genomic DNA sequences of 1069 base pairs in the NS1/VP1 region showed complete identity between the patient and donor samples.