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CASE REPORT

Parvovirus B19 Infection after Plasma Exchange for Myasthenia Gravis

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ABSTRACT

We describe a case of pure red cell aplasia caused by a B19 parvovirus infection in a female myasthenic patient treated with plasma exchange, corticosteroids, and cholinesterase inhibitors. Two weeks after albumin infusion, she developed anemia with an absence of reticulocytes. A bone marrow aspirate was performed, showing a markedly hypoplastic erythroid series with numerous giant pronormoblasts. Anemia with severe reticulocytopenia and morphology of bone marrow suggested a diagnosis of pure erythroblastopenia due to parvovirus B19 infection, which was confirmed by positive immunoglobulin (Ig)M and IgG anti-B19 virus. The patient successfully responded to IVIG treatment with a complete remission. In this case, we could not confirm whether an albumin-derived infection combined with a concomitant immunocompromised condition due to myasthenia and immunosuppressive treatment was responsible for the disease. Although human B19 DNA content does not reflect infectivity, it is not possible to exclude that blood derivates, such as albumin, clot factors, and immune globulin may be infectious. Actually, blood component B19 infection is still an unresolved problem. Many strategies such as new methods for viral inactivation and discarding positive B19 units may help to increase blood product safety. *Lab Hematol* 2007;13:34-38.

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KEY WORDS: Parvovirus B19 • Pure red cell aplasia • Albumin • Myasthenia gravis • Plasma exchange

INTRODUCTION

Parvovirus B19 is a single-stranded DNA virus, forming small capsides and lacking a lipid envelope. Its genome encodes 3 major viral proteins, VP1 and VP2, the viral capsid proteins, which lead to self-assembly of viral particles, and NS1, a nonstructural protein, which is responsible for cytotoxicity. It has a peculiar tropism for human erythroid progenitors, with inhibition of erythroid colony growth and cytopathic effect [1-2].

B19 parvovirus is a common infection in humans, and about 50% of adults have immunoglobulin (Ig)G antibodies against the virus. Parvovirus infection is common in childhood and continues at a low rate throughout adult life. Most cases of parvovirus infection are asymptomatic. The most common clinical presentation is fifth disease of childhood, characterized by typical exanthema, fever, and flu-like symptoms. Acute or chronic arthropathy due to deposition of immune complexes may occur in adults. In patients with chronic hemolytic anemia, such as hereditary spherocytosis and sickle cell disease, acute parvovirus B19 infection can cause an abrupt cessation of red cell production, with transient aplastic crisis. In patients with immunodeficiency states, such as congenital immunodeficiencies or AIDS and patients receiving cytotoxic chemotherapy or immunosuppressive drugs, such as administered after an organ transplantation, there can be a failure to produce neutralizing antibodies. In these cases, pure red cell apla-

sia can develop, with an absence of circulating reticulocytes and giant pronormoblasts in the bone marrow, without maturing normoblasts. Hydrops fetalis from transplacental infection and usually transitory hemophagocytic syndrome are other clinical disorders caused by B19 [3].

Parvovirus B19 transmission by blood products and plasma derivates, such as albumin, clotting factor concentrates, and intravenous immunoglobulin (IVIG) has been repeatedly demonstrated [4]. Transmissibility in coagulation products has occurred among patients who received heat-treated, pasteurized, monoclonally purified and solvent-detergent–treated concentrates [5]. Infection with B19 due to transfusion with cellular blood products is a rare event, but it has been reported twice with red blood cells and once with platelets [6-8]. We report a case of a myasthenic patient with pure red cell aplasia due to a parvovirus B19 infection.

CLINICAL CASE DESCRIPTION

In 1997, a 29-year-old woman complained of intermittent speaking difficulty (dysarthria). In April 1998, 10 days before the full-term delivery of her second healthy baby, more severe symptoms appeared, such as facial nerve and oro-pharyngeal deficit and weakness of the arms and legs. Ten days after delivery, the patient was admitted to a hospital for a typical myasthenic crisis with severe weakening of respiratory muscles, requiring a respirator to assist ventilation. Treatment was started with 4 consecutive plasma exchanges and administration of corticosteroids and cholinesterase inhibitors (pyridostigmine bromide) with marked clinical improvement. In August 1998, the patient withdrew from medical therapy, which led to a worsening of symptoms and a new hospitalization in a different institution. There she was treated with 5 therapeutic plasma exchanges using albumin as replacement fluid. Medical treatment was started again. On August 31, she had a deep vein thrombosis, treated with IV heparin. On September 3, she was admitted to the Neurology Department of our hospital. At admission, the patient had normochromic-normocytic anemia (hemoglobin [Hgb], 97 g/L), with normal platelet and white blood cell counts.

Two weeks later, anemia worsened and was associated with thrombocytopenia (Hgb, 81 g/L; platelets, 57×10^9/L) (Figure 1). Schistocytes were absent. A diagnosis of heparin-induced thrombocytopenia was made. Heparin tapering was started, and the platelet count improved. A few days later, since anemia was still severe (Hgb, 80 g/L) and of an aregenerative type with an absence of reticulocytes, a bone marrow aspirate was performed. This showed many moderate hypercellular marrow particles and an increased number of megakaryocytes. An erythroid series was markedly hypoplastic with complete maturative arrest. The only visible erythroid precursors were giant pronormoblasts with vacuolated deep basophilic cytoplasm, sometimes grouped in clusters simulating metastatic cells (Figures 2 and 3). Anemia with severe reticulocytopenia and morphology of bone marrow suggested a diagnosis of pure erythroblastopenia due to parvovirus B19 infection, which was confirmed by positive tests for IgM and IgG anti-B19 virus. Increased megakaryocytes tended to confirm that thrombocytopenia was heparin-induced. The patient was treated with immune globulin (0.4 g/kg for 4 days). Reticulocytosis appeared on September 30 (202×10^9/L; normal values, 30-90 $\times 10^9$/L). Anemia recovered slowly (Hgb, 92 g/L at discharge), and thrombocytopenia completely regressed. The patient was admitted again to

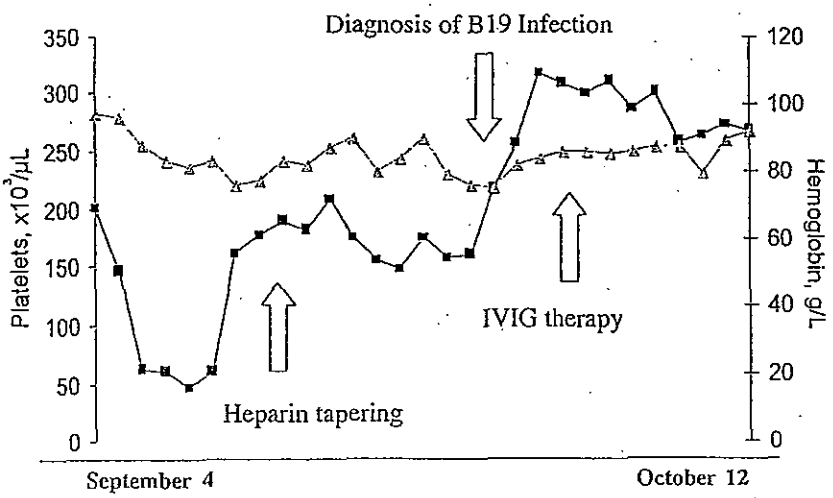

FIGURE 1. Hematological values and clinical course of the patient from admission (September 4, 1998) to discharge (October, 12 1998). Triangle indicates platelet count; square, hemoglobin concentration.

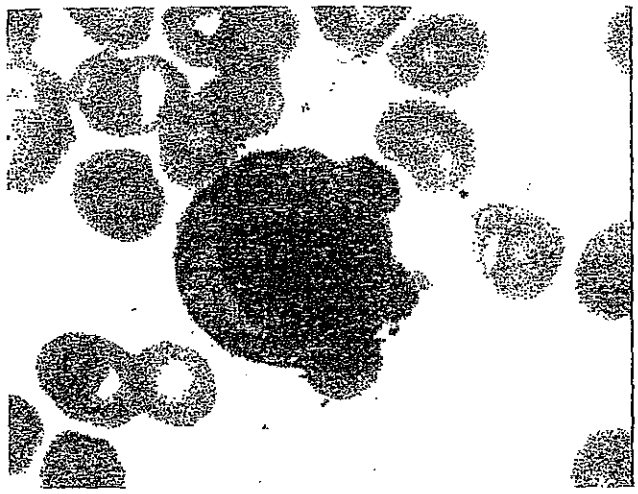

FIGURE 2. Basophilic giant pronormoblast with pseudopodia or "dog ears."

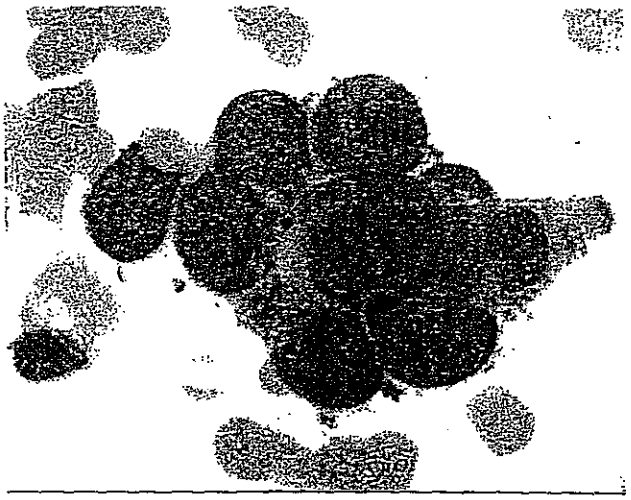

FIGURE 3. A cluster of pronormoblasts with maturative arrest.

the hospital in May 1999 for surgical resection of a thymoma. At that time, her full blood count was normal, IgM anti-B19 was negative, and IgG anti-B19 was still positive.

DISCUSSION

We described a case of pure red cell aplasia caused by parvovirus B19 in a patient with myasthenia gravis treated with plasma exchanges using albumin, corticosteroids, and cholinesterase inhibitors.

Parvovirus B19 has a particular tropism for erythroid progenitors. The cellular receptor for B19 is erythrocyte P antigen, a globoside that consists of a long-chain fatty acid on a ceramide back-bone structure with 4 sugar residues ending with terminal N-acetyl galactosamine. The P antigen is a common erythrocyte and erythroblast antigen, and it is expressed in almost all subjects. People who lack the P antigen are resistant to infection [1]. In this case, the patient had P_1 phenotype, which is the most common phenotype among Caucasians (79%) and Africans (94%). P_2 phenotype is more common among Asian people, such as Cambodians and Vietnamese. [9].

P antigen is also expressed on megakaryocytes, endothelial cells, synovium, villous trophoblast cells of placental tissues, fetal liver, and heart cells. B19 infection may also be responsible for thrombocytopenia, and megakaryocytes may be lysed by restricted expression of viral proteins in the absence of viral propagation [10]. In this case, thrombocytopenia was heparin-induced, confirmed by an increase of the peripheral platelet count when heparin tapering was started (Figure 1). Heparin-induced thrombocytopenia is more often reported after orthopedic, cardiac, or vascular surgery, but it may develop in any patient exposed to unfractionated heparin or low molecular weight heparin [11]. Furthermore, the patient's bone marrow showed increased megakaryocytes, which tended to confirm that thrombocytopenia was heparin induced.

After binding with P antigen, the virus enters the targeted cells, probably because of the VP1 phospholipase activity, and starts to synthesize viral components. It has been demonstrated that B19 is a potent inhibitor of erythroid cell differentiation, and it is cytotoxic for erythroid precursors. It acts by inducing apoptosis through the activation of the caspase pathway or direct lytic effect on erythroid cells. Apoptosis is mediated by NS1 expression, which induces activation of caspase-3, caspase-6, and caspase-8 in a cellular model [12,13].

The virus is also responsible for a cytopathic effect on cells causing a maturative arrest in the erythroid cell line. In smears from bone marrow aspirate, the pathognomonic cell for B19 infection is the giant proerythroblast, which is a large cell, from 25 to 32 μm in diameter, with a high nucleo-cytoplasmic ratio; the nucleus is round and it has a fine and uncondensed chromatin pattern with irregular, indistinct purple-colored inclusions. A giant proerythroblast has a dark blue vacuolated cytoplasm with small broad-based cytoplasmic pseudopodia, named "dog-ear" projections. Sometimes they are grouped in clusters simulating metastatic cells [14]. As shown in Figures 2 and 3, the patient's bone marrow was characterized by the presence of large numbers of these immature erythroid cells. This accounts for anemia with severe reticulocytopenia, sometimes requiring red blood cell transfusions.

In patients with chronic hemolytic disorders, such as sickle cell disease and spherocytosis, B19 may cause transient aplastic crisis characterized by aregenerative acute anemia, sometimes associated with pancytopenia. Persisting B19 infection can occur in a wide variety of conditions, including congenital immunodeficiencies, HIV infection, lymphoproliferative disorders, and transplantation. In these cases, patients may have chronic pure red cell aplasia and more

rarely pancytopenia [15]. In pregnant women, parvovirus B19 may be transmitted to the fetus and may lead to miscarriage or hydrops fetalis [16].

Although the presence of giant proerythroblasts is suggestive of B19 infection, the diagnosis should be made by serological detection of antibodies or molecular detection of viral components. Serological determination of antibodies may be performed by enzyme-linked immunosorbent assays that are able to identify IgM and IgG antibodies. IgM antibodies remain detectable for 2 or 3 months following the infection, as opposed to IgG antibodies which appear 2 weeks after the infection but persist for life. Immunocompromised patients sometimes are not able to produce IgM, and in these cases molecular tests, such as direct hybridization and gene-amplification methods, may be helpful to confirm a clinical suspicion [2].For our patient, tests gave positive results for IgG and IgM at the time of the diagnosis. Some months later, because of a further admission, her test results for IgM anti-B19 were negative, while those for IgG anti-B19 were still positive. At that time, molecular tests were not performed.

In children and immunocompetent adults, B19 infection does not require any treatment. In patients with immunodeficiencies or pure red cell aplasia, treatment with IVIG may be helpful and should be associated with discontinuing immunosuppressive drugs. Generally a 5- or 10-day course of IVIG (0.4 g/kg of body weight) causes a rapid virus elimination associated with reticulocytosis and elevation of Hgb concentration [17].

B19 may be transmitted by respiratory droplets, but secondary infection among households and nosocomial infection have been described [18,19]. B19 transmission by blood products and derivates, such as IVIG [20], solvent-detergent–treated pooled plasma [21], and clotting factor concentrates [5] has been repeatedly demonstrated, even after viral inactivation methods.

B19 is an envelope-free virus and therefore resistant to solvent-detergent treatment. This treatment is effective for clearance of HBV, HCV, and HIV, but it is not effective for HAV and B19, both of which lack the envelope. B19 resistance to heat is controversial. The virus is relatively heat stable [21], but Blümel et al [22] showed that pasteurization for 10 hours at 60°C rapidly inactivates B19. Although human B19 DNA content does not reflect infectivity, we cannot exclude the possibility that blood derivates, such as albumin, clot factors, and immune globulin may be infectious. In our patient, we could not confirm whether an albumin-derived infection combined with a concomitant immunocompromised condition due to myasthenia and immunosuppressive treatment was responsible for the disease. Blood component B19 infection is still an unresolved problem. Many strategies such as new methods for viral inactivation and discarding positive-B19 units [23-25] may help to increase blood product safety.

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医薬品 研究報告 調査報告書

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研究報告の概要

○マレーシアから帰国したヨーロッパ人旅行者におけるサルマラリア
2007年にマレー半島でフィンランドの旅行者が*Plasmodium knowlesi*に感染した。
患者は53歳男性で、マレー半島を4週間旅行してフィンランドに帰国した3日後に高熱を発症し、翌日受診した。患者ははじめの2週間クアラルンプールに滞在し、周辺地域を数日間旅行した。その後自動車で北西の海岸部に向かい5日間イポー近くのジャングルで過ごした。この間蚊帳のない家に泊まり防虫剤は使用していなかったが、蚊に刺されたという報告はなかった。最後の週はランカウイ・ビーチの高級ホテルに滞在していた。
血液塗抹検査でマラリア原虫が陽性となり、入院後塩酸キニーネとドキシサイクリンを合計10日間投与された。回復後12ヶ月間のフォローアップ期間中に再発は見られなかった。PCR産生物のヌクレオチド配列解析を行ったところGenBankに登録されていた*P. knowlesi*と一致した。
*P. knowlesi*は通常サルにマラリアを引き起こす寄生虫であるが、ヒトマラリアを引き起こす可能性がある第5のマラリア原虫(*Plasmodium* species)とされている。当該疾患はヒトの生命を脅かす恐れがあり、臨床医や臨床検査技師は、旅行者の当該病原体についての認識を高めるべきである。

使用上の注意記載状況・その他参考事項等

解凍赤血球濃厚液「日赤」
照射解凍赤血球濃厚液「日赤」
解凍赤血球-LR「日赤」
照射解凍赤血球-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
2007年にマレー半島でフィンランドの旅行者が、通常サルにマラリアを引き起こす*Plasmodium knowlesi*に感染し、帰国後に発症したとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、マラリア流行地への旅行者または居住経験者の献血を一定期間延期している(1～3年の延期を行うとともに、帰国(入国)後マラリアを思わせる症状があった場合は、感染が否定されるまでの間についても献血を見合わせる)。今後も引き続き、マラリア感染に関する新たな知見及び情報の収集、対応に努める。

(12)

Monkey Malaria in a European Traveler Returning from Malaysia

Anu Kantele, Hanspeter Marti, Ingrid Felger, Dania Müller, and T. Sakari Jokiranta

In 2007, a Finnish traveler was infected in Peninsular Malaysia with *Plasmodium knowlesi*, a parasite that usually causes malaria in monkeys. *P. knowlesi* has established itself as the fifth *Plasmodium* species that can cause human malaria. The disease is potentially life-threatening in humans; clinicians and laboratory personnel should become more aware of this pathogen in travelers.

Traditionally, only 4 *Plasmodium* species have been known to cause malaria in humans: *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*, although >26 *Plasmodium* species are known to circulate among primate populations (*1*). Some of these species have been implicated in symptomatic human malaria after experimental or accidental infection (*2*). Only a few reports of naturally acquired monkey malaria in humans are currently available (*1,3–9*). The lack of data may be because light microscopy has been used as the sole diagnostic method and an atypical *Plasmodium* species may have been misidentified as one of the 4 traditional *Plasmodium* species causing human malaria.

P. knowlesi was first described in 1931 in a long-tailed macaque imported from Singapore to India; in 1932, *P. knowlesi* was experimentally shown to be infectious to humans (*10*). The first natural infection of *P. knowlesi* in humans was reported in 1965 in a man returning to the United States after a visit to Peninsular Malaysia (*11*). Subsequently, in 1971, there was a report of a presumed natural infection in a citizen of Malaysia (*6*). Despite extensive studies in Malaysia in the 1960s (*2*), no other reports were published on naturally acquired *P. knowlesi* infections in humans until 2004, when Singh et al. studied PCR-negative *P. malariae* cases in the Kapit division in Sarawak, Malaysia (*3*). A different PCR analysis showed that *P. knowlesi* caused 58% of the 208 malaria cases studied. Further cases reported from China (*4*), Thailand (*5*), Philippines (*8*), and Singapore (*12*) show that *P. knowlesi* infections in humans are not found exclusively in Malaysia. Recently, Cox-Singh et al. reported that *P. knowlesi* is widely distributed among inhabitants of Malaysia (*7*).

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The Study

A 53-year-old Finnish man was admitted to a local hospital in Finland in March 2007 with fever after 4 weeks of travel in Peninsular Malaysia. He had not taken any antimalarial prophylaxis. In Malaysia, he spent 2 weeks in Kuala Lumpur and made a few day trips to surrounding rural areas. Thereafter, he traveled by car to the northwestern coast and stayed for 5 days in the jungle ≈80 km south of Ipoh. While in this area, he slept in a house without mosquito screens or nets and did not use any repellents; he did not report any mosquito bites. The last week of his travel was spent in the Langkawi Beach area where he stayed at a high-quality hotel. During his trip he occasionally had some minor abdominal problems, but these symptoms subsided spontaneously after his return to Finland. High fever (38.8°C axillary temperature) occurred 3 days after his return to Finland but abated quickly. On the fourth day, the fever returned and he sought medical care at a local hospital. Laboratory tests showed the following results: C-reactive protein 2.0 mg/dL (normal range <1.0 mg/dL), hemoglobin 15.2 g/dL (normal range 13.4–16.7 g/dL), leukocyte count 2.6 × 10^9/L (normal range 3.4–8.2 × 10^9/L), and thrombocytes 143 × 10^9/L (normal range 150–360 × 10^9/L). Blood smear was positive for *Plasmodium* organisms, and the causative agent was identified as *P. falciparum* with levels of parasitemia <1.0%. The patient was admitted to the hospital and given intravenous (IV) quinine dihydrochloride and oral doxycycline.

On day 2 of the patient's hospital stay, fever returned and he was transferred to the Helsinki University Central Hospital (Department of Infectious Diseases at Aurora Hospital). Blood smears obtained there showed *Plasmodium* parasites that were considered atypical, and the laboratory reported suspicion of a co-infection (*P. falciparum* and *P. malariae*) (Figure). The IV quinine dihydrochloride was replaced with oral quinine hydrochloride, and doxycycline was continued. During treatment, the patient experienced an attack of hypoglycemia (electrocardiogram and blood pressure was normal during this attack), transient mild visual and hearing loss, and transient lymphopenia (a low of 0.46 × 10^9/L). He received quinine hydrochloride and doxycycline for a total of 10 days.

Because identification of the *Plasmodium* species was difficult, a blood sample was drawn for PCR analysis on day 2 of hospitalization. First, a nested PCR was performed according to a standard protocol with rOval and rPLU2 primers (template DNA purified in Basel from 200 μL of erythrocytes by QIAamp DNA Mini Blood Kit (QIAGEN,