

HUMAN PARVOVIRUS B19 INFECTION PRESENTING AS PERSISTENT ANEMIA IN RENAL TRANSPLANT RECIPIENTS

SRINIVAS PAMIDI,¹ KENNETH FRIEDMAN,² BAL KAMPALATH,³ CAMELLIA ESHOA,³ AND SUNDARAM HARIHARAN^{4,5}

Department of Internal Medicine, Division of Hematology, Department of Pathology, and Division of Nephrology, Medical College of Wisconsin; and The Blood Center of Southeastern Wisconsin, Milwaukee, Wisconsin

Background. Immunosuppression cannot be achieved without immunosuppressive effects. Human Parvovirus infection is known to occur after organ transplantation. We present our experience with Parvovirus infection in two cases.

Methods and Results. Two kidney transplant recipients developed symptomatic anemia requiring blood transfusions. Common causes of anemia, such as gastrointestinal bleeding, iron/vitamin deficiencies, hemolysis, and drug toxicities, were ruled out. A peripheral smear revealed low reticulocyte count. Bone marrow examination showed hypoplastic bone marrow with intranuclear inclusions suggestive of human Parvovirus. This was confirmed by immunohistochemical analysis. Treatment with i.v. immunoglobulin G resulted in a dramatic sustained response. Transplant kidney function remained stable.

Conclusion. Human Parvovirus infections should be considered in immunosuppressed individuals with anemia with poor bone marrow response. Bone marrow examination can reveal viral inclusions and can be confirmed by immunohistochemical analysis. Intravenous immunoglobulin G results in resolution of anemia.

Human Parvovirus (HPV) B19 is a small, icosahedral, nonenveloped, single stranded DNA virus, and it is the only parvovirus that is known to be a human pathogen (1). This virus has tropism for human erythroid precursor cells, with lysis of infected erythroblasts within the marrow (2, 3). This causes significant anemia with reticulocytopenia (4). In immunocompetent hosts, this anemia is generally transient and mild; however, it may be severe in patients with sickle cell anemia (4). In immunosuppressed individuals, the anemia may be persistent and severe because they cannot mount humoral antibodies to the infectious agent (5). In this report, we present our experience with two cases of persistent anemia secondary to parvovirus in immunosuppressed renal transplant recipients.

A 46-year-old white man with end stage renal disease (ESRD) secondary to Henoch Schonlein purpura underwent a cadaveric renal transplant with 2DR,1B match in September 1997. Other medical problems included hypertension, obesity, and protein S deficiency. The posttransplantation course was unremarkable, with a stable serum creatinine of

1.6 mg/dl and hematocrit of 43%. He did not experience any rejection episodes. Medications included posttransplantation immunosuppressive therapy with mycophenolate mofetil (MMF) 500 mg b.i.d., prednisone 10 mg q.d., tacrolimus 11 mg b.i.d., warfarin 4 mg q.d. for protein S deficiency, procardiol 30 mg b.i.d., enalapril 10 mg b.i.d. for hypertension, and sulfamethoxazole/trimethoprim 1 tablet 3×/week for pneumocystic prophylaxis. One year posttransplantation, he presented with a 2-month history of progressive dyspnea on exertion, fatigue, and lightheadedness when the diagnosis of anemia was made. His laboratory findings were the following: white blood cell count $8.3 \times 10^3/\text{mm}^3$; red blood cell (RBC) count $2.47 \times 10^6/\text{mm}^3$; hemoglobin 7.6 g/dl; hematocrit 22.3%; mean corpuscular volume $90 \mu\text{m}^3$; red cell distribution width 20.4%; platelet count $257 \times 10^3/\text{mm}^3$; reticulocytes 0.59% (absolute reticulocyte count $0.0144 \times 10^6/\text{mm}^3$); serum folate 17 ng/ml; serum vitamin B12–479 pg/ml; serum ferritin–847 ng/ml; and erythropoietin 340 U. The red cell morphologic findings in the peripheral blood was normocytic-normochromic, with decreased reticulocyte response. The gastrointestinal work-up, including upper endoscopy and colonoscopy, were negative. Anemia persisted, despite the discontinuation of enalapril. The bone marrow demonstrated an increased cellularity with slightly decreased myeloid to erythroid ratio because of erythroid hyperplasia. The erythroid hyperplasia was confirmed by immunoperoxidase stain, glycophorin A (Dako, Carpinteria, CA). The erythroblasts displayed all stages of maturation, with a shift toward immaturity. Mild dyserythropoiesis was evident with occasional cells exhibiting binucleation, nuclear budding, and nucleocytoplasmic asynchrony. In the bone marrow aspirate, proerythroblasts were enlarged with deep basophilic cytoplasm and poorly demarcated intranuclear inclusions (Fig. 1). Intranuclear eosinophilic inclusions in erythroid series were also detected from a bone marrow trephine biopsy specimen. The intranuclear inclusions were confirmed by immunoperoxidase stain against parvovirus B19 (Dako, Carpinteria, CA) (Fig. 2) to result from Parvovirus. The granulocytic series and megakaryocytes did not show any hypoplastic change. The patient received 16 U of packed RBC over 6 weeks, with continued anemia without reticulocytosis. Immunoassay for Parvovirus revealed immunoglobulin (Ig)M positivity (> 1:10) and equivocal IgG. The diagnosis of Parvovirus induced red cell aplasia was made serologically as well as with bone marrow findings, and the patient was given i.v. IgG 50 g q.d. for 5 days. This resulted in a dramatic improvement in his hematocrit. His hematocrit remained stable for 14 months after the immunotherapy. In February 2000, his hematocrit was 42%, and parvovirus titer (IgG) > 1:64.

A 40-year-old white woman with ESRD secondary to

¹ Department of Internal Medicine.

² Division of Hematology and The Blood Center of Southeastern Wisconsin.

³ Department of Pathology

⁴ Division of Nephrology.

⁵ Address correspondence to: Sundaram Hariharan, M.D., Medical College of Wisconsin, Division of Nephrology, 9200 W. Wisconsin Ave. Milwaukee, WI 53226. E-mail: hari@mcw.edu.

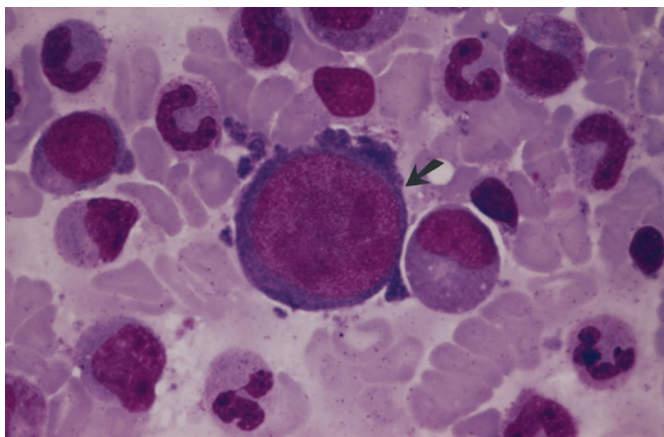


FIGURE 1. Bone marrow aspirate showing greater erythroblast with poorly demarcated intranuclear parvovirus inclusions (arrow) ($\times 350$ Wright-Giemsa).

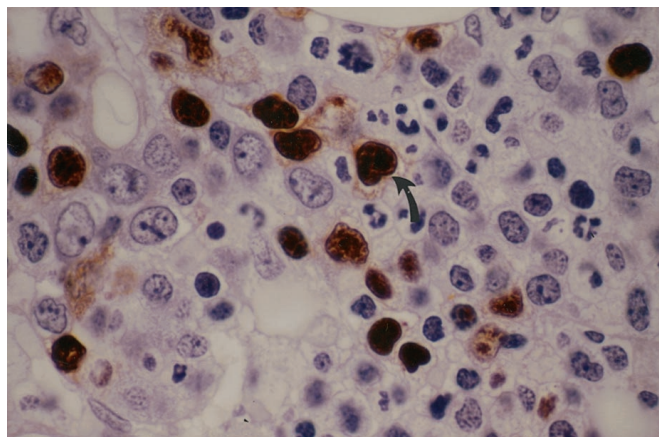


FIGURE 2. Immunohistochemical stains using monoclonal antibodies against parvovirus highlight intranuclear inclusions (arrow) in erythroid precursors ($\times 250$).

Hemolytic uremic syndrome/Thrombotic thrombocytopenic purpura. She received a 6-antigen matched second renal transplant in January 1999. She lost her earlier transplant because of recurrent Hemolytic uremic syndrome/Thrombotic thrombocytopenic purpura. She presented a month after her second transplant with shortness of breath and tiredness. Her hematocrit had decreased from 35% to 23% over 4 weeks. The posttransplantation renal function was normal, with a creatinine of 1.2 mg/dl. Medications included tacrolimus 7 mg b.i.d., prednisone 25 mg b.i.d., MMF 500 mg b.i.d., oral ganciclovir, amlodipine, atenolol, and sulfamethoxazole/trimethoprim. Anemia persisted despite the discontinuation of ganciclovir and MMF. Laboratory findings were the following: white blood cell count $6.8 \times 10^3/\text{mm}^3$; RBC count $3.08 \times 10^6/\text{mm}^3$; hemoglobin 7.8 g/dl; hematocrit 23%; mean corpuscular volume $94.0 \mu\text{m}^3$; red cell distribution width 15.6%; platelet count $336 \times 10^3/\text{mm}^3$; reticulocytes 0.45% (absolute reticulocyte count $0.0141 \times 10^6/\text{mm}^3$); serum folate $>12 \text{ ng/ml}$; serum vitamin B12–512 pg/ml; serum ferritin 1000 ng/ml; and erythropoietin 251 U. A gastric endoscopy and colonoscopy ruled out gastrointestinal bleeding. A peripheral blood smear revealed a decreased RBC count with normocytic-normochromic erythrocytes and decreased reticulocyte response. The bone marrow aspiration demonstrated slight hypocellularity with marked increase in the myeloid to erythroid ratio because of profound erythroid hypoplasia. The erythroid series displayed lack of maturation, with a predominance of proerythroblasts. In the bone marrow aspirate, the proerythroblasts were giant with dense basophilic cytoplasm, delicate chromatin, and ill defined intranuclear inclusions (as in Figure 1, case 1). Intranuclear eosinophilic inclusions in erythroid series were detected from bone marrow trephine biopsy specimens, which stained positive for Parvovirus by immunoperoxidase stain (Dako, Carpinteria, CA) (as in Figure 2, case 1). The granulocytic and megakaryocytic series were unremarkable. Bone marrow aspiration revealed similar intranuclear inclusions, such as in case 1. This was also confirmed with immunohistochemical staining. Immunoassay of HPV B19 showed absent IgG and equivocal IgM. The patient was given a 5-day course of i.v. IgG 25 g q.d. for Parvovirus-induced red cell aplasia. The patient's anemia

improved after immunotherapy (Fig. 3) and has remained stable since then. Her last hematocrit, in February 2000, was 48%, and the parvovirus titer (IgG) was $> 1:64$.

HPV B19 has a tropism for human erythroid progenitor cells and multiplies and then induces cell lysis (2). The pathogenesis of HPV B19 is biphasic in immunocompetent individuals. The 1st phase is characterized by viremia for about a week after exposure to the virus. Nonspecific symptoms, such as malaise, headache, myalgia, and pruritus, may be associated with anemia. Viral clearance is correlated with the development of IgM antibodies, usually first detectable about 10 days after infection, and may persist for a few months. IgG antibodies develop at the end of the 2nd week and persist indefinitely. The second phase of infection begins around 15–17 days after challenge and is manifested by erythematous rash, arthritis, and pruritus (6). Anemia resolves after the second phase.

Immunocompetent patients usually manifest with mild asymptomatic anemia. However, anemia may be severe in patients with a pre-existing chronic hemolysis process, such as sickle cell anemia (4). In an immunosuppressed individual, this infection results in a persistent anemia as a result of inadequate humoral immune response, resulting in persistent reticulocytopenia with anemia (5, 7). HPV B19 virus infection with persistent anemia has been reported in organ transplant recipients receiving immunosuppressive therapy (7, 8, 9).

This report illustrates HPV-B19 infection with manifestation of anemia in renal transplant recipients. The diagnosis of HPV infection can be made by the demonstration of B19 IgM antibodies in immunocompetent patients (10). The ELISA technique for IgG and IgM antibodies and polymerase chain reaction for antigen detection can be diagnostic (11). Electron microscopic analysis has also helped to identify HPV B19 virus from bone marrow in some cases (12). However, diagnosis of HPV B19 infection is more difficult to diagnose in an immunocompromised patient because IgM and IgG may be absent or the titre may not be significantly elevated. Our case 1 had documented IgM antibody production with failure of IgG antibody synthesis with persistent anemia. However, case 2 never augmented IgM antibody

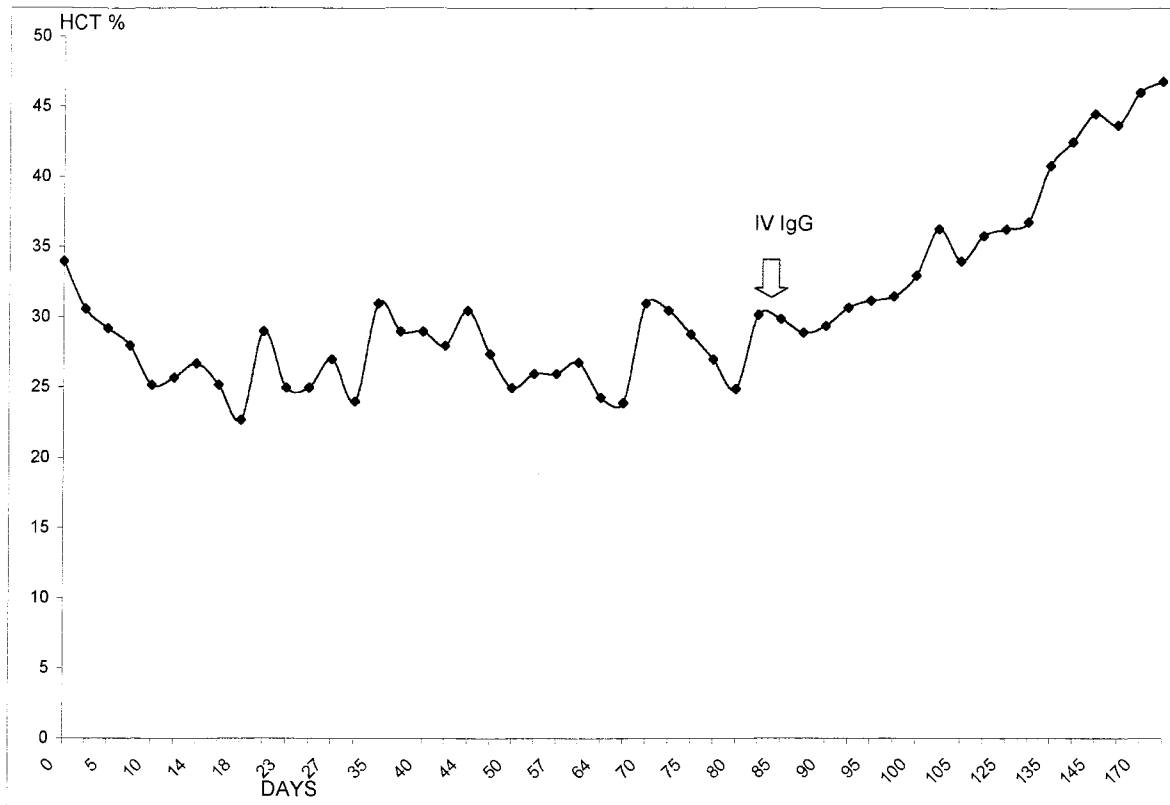


FIGURE 3. The hematocrit pre- and post-i.v. IgG treatment in case 2.

production, and the virus was confirmed by bone marrow examination. In both cases, the diagnosis of this condition was delayed, and patients received blood transfusions and other common causes of anemia were ruled out. In case 2, the erythroid hypoplasia and lack of maturation beyond proerythroblasts provided a clue for possible parvoviral etiology, which led to the search for parvoviral inclusions and confirmatory immunostain for parvoviral inclusions. In case 1, however, the erythroid series were hyperplastic and exhibited full spectrum of maturation and dyspoietic changes. Concomitant dyspoietic changes in megakaryocytic series added to the confusion, and a myelodysplastic process was the major differential diagnosis. In this case, positive serologic findings for parvovirus were the major clue prompting performance of the confirming immunostain for parvovirus.

Intravenous IgG has been used to treat patients with this infection who are immuno-compromised (8, 9). Reduction in immunosuppression may also be helpful as an approach to treat this infection. Recurrence of infection has also been reported and can be treated appropriately with i.v. IgG. HPV B19 cell lines have been developed and the role of immunization needs further evaluation. Both of our patients responded very well, with a dramatic improvement after the administration of i.v. IgG treatment. Both of these recipients have a hematocrit >40%, with resolution of all symptoms and with an elevated IgG titer, suggesting immunity.

In conclusion, HPV infection should be considered a differential diagnosis of persistent anemia in transplant recipi-

ents. Reticulocytopenia and bone marrow erythroid hypoplasia should raise suspicion of this infection. The detection of IgG, IgM antibodies, and marrow histologic findings should raise suspicion and can be confirmed by demonstrating the virus with a specific monoclonal antibody stain to HPV B19. Treatment with i.v. IgG should resolve aplastic anemia.

REFERENCES

1. Siegl G, Bates RC, Berns KI, et al. Characteristics and taxonomy of parvoviridae. *Intervirology* 1985; 23: 61.
2. Young N, Harrison M, Moore J, et al. Direct demonstration of the human parvovirus in erythroid progenitor cells infected in vitro. *J Clin Invest* 1989; 74: 2024.
3. Brown KE, Anderson SM, Young NS. Erythrocyte P antigen: cellular receptor for B19 parvovirus. *Science* 1993; 262: 114.
4. Goldstein AR, Anderson MJ, Serjeant GR, et al. Parvovirus associated aplastic crisis in homozygous sickle cell disease. *Arch Dis Child* 1987; 62: 585.
5. Frickhofen N, Abkowitz JL, Safford M, et al. Persistent B19 parvovirus infection in patients infected with human immunodeficiency virus type1(HIV-1): a treatable cause of anemia in AIDS. *Ann Intern Med* 1990; 113: 926.
6. Portmore AC. Parvoviruses (Erythema infectiosum, aplastic crisis). In: Mandell GL, Bennett JE, Dolin R, eds. *Principles & practice of infectious diseases*, 4th edition. Churchill Livingstone, New York. 1995: 1439.
7. Neild G, Anderson M, Hawes S, et al. Parvovirus infection after renal transplant. *Lancet* 1986; 2: 1226.

8. Moudgil A, Shidban H, Nast CC, et al. Parvovirus B19 infected-related complications in renal transplant recipients: treatment with intravenous immunoglobulin. *Transplantation* 1997; 64: 1847.
9. Marchand S, Tchernia G, Hiesse C, et al. Human parvovirus B19 infection in organ transplant recipients. *Clin Transplant* 1999; 13: 17.
10. Anderson MJ, Davis LR, Jones SE, et al. The development and use of an antibody capture radioimmunoassay for specific IgM to a human parvovirus like agent. *J Hyg Epidemiol Microbiol Immunol* 1982; 88: 309.
11. Sevall JS, Ritenhouse J, Peter JB, et al. Laboratory diagnosis of parvovirus B19 infection. *J Clin Lab Analysis* 1992; 6: 171.
12. Knisely AS, O'Shea PA, McMillan P, et al. Electron microscopic identification of parvovirus virions in erythroid-line cells in fetal hydrops fetalis. *Pediatr Pathol* 1988; 8: 163.

Received 27 December 1999.

Accepted 29 March 2000.