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CASE REPORT

Parvovirus B19-induced anemia in renal transplantation: a role for rHuEPO in resistance to classical treatment

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Summary

Human parvovirus B19 (PVB 19) is responsible for pure red cell aplasia in immunocompromised patients, and particularly solid organ recipients. Intravenous immunoglobulins (IVIG) have been shown to be efficient to achieve the correction of anemia in association with the reduction of immunosuppression. We report a case of kidney transplant recipient with PVB 19-induced anemia that did not respond to recombinant human erythropoietin (rHuEPO) and to a first course of IVIG. After discontinuation of rHuEPO, a second course of IVIG was successful with the resolution of anemia. We discuss the role of rHuEPO that may facilitate PVB 19 replication in erythropoietin-sensitive human erythroid progenitor cells.

Introduction

Severe anemia frequently occurs in organ transplant recipients, and is particularly associated with the use of immunosuppressive drugs and with some infectious agents. An increasing number of reports suggest that human parvovirus B19 (PVB 19) is responsible for pure red cell aplasia in immunocompromised patient [1,2]. In the study by Cavallo *et al.* [3], up to 23% of kidney transplant recipients with anemia were actively infected with PVB 19. The viral replication was detected by polymerase chain reaction (PCR) in the serum. In 90% of these cases, the infection was caused by a viral reactivation and in 10% to a primo-infection. This complication usually responds to a reduction of immunosuppression or to intravenous globulins (IVIG) [4]. In the case of severe anemia, red blood cell formation is commonly stimulated with recombinant human erythropoietin (rHuEPO) to avoid the need for red cell transfusion. PVB 19 targets and replicates in erythropoietin-sensitive human erythroid progenitor cells [5] and then rHuEPO may favor chronic PVB 19 infection. We report the case of a kidney transplant recipient with PVB 19-induced anemia that did not respond to rHuEPO and to classical treatments. The role of rHuEPO is discussed.

Case report

A 51-year-old woman was on maintenance hemodialysis for 2 years because of an unknown nephropathy. Hemoglobin concentration was stable at 11 g/dl without rHuEPO therapy. In April 2001, she received a first cadaveric renal transplant. The immunosuppressive regimen consisted of tacrolimus, mycophenolate mofetil (MMF) and prednisolone. Serum creatinine was 100 μmol/l 2 weeks post-transplant, and the tacrolimus trough level ranged between 8 and 12 ng/ml. One month after transplantation, she developed severe anemia (serum hemoglobin drop to 6.3 g/dl) following a flu-like syndrome. Reticulocyte count was 10×10^9/l (0.4% of total blood red cells).

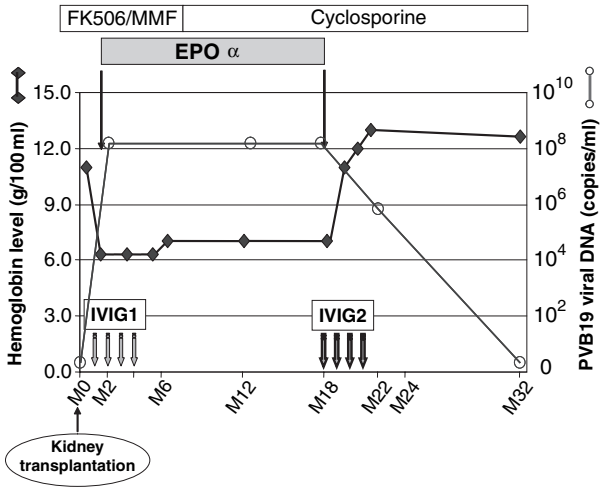

Figure 1 Evolution of parvovirus B19-induced anemia.

Ferritin and vitamin B12 values were normal. Platelets, white blood cell counts and hepatic tests were normal. Serum erythropoietin concentration was 37 mIU/ml. Treatment with subcutaneous rHuEPO (Epoetin α) was initiated (9000 IU/week). Despite rHuEPO therapy, the hemoglobin concentration and the reticulocyte count remained low, and the patient required transfusion of three red cell units per month. Anti-erythropoietin antibodies were not found in the serum. The patient was negative for cytomegalovirus pp65 antigens. No anti-PVB 19 IgM or IgG antibodies were detected in sera. In contrast, quantitative PCR using specific primers for PVB 19 was positive in blood sample with more than 10^8 copies/ml (see Fig. 1). The diagnosis of pure red cell aplasia because of the primary PVB 19 infection was made.

Intravenous polyvalent immunoglobulins were administered at 0.5 g/kg/day every 4 weeks for 4 months. No correction of anemia was noted. The quantitative PCR for PVB 19 was still above 10^8 copies/ml in the serum. The dose of subcutaneous rHuEPO was increased to 15 000 U/week, MMF was discontinued and tacrolimus was replaced by cyclosporine.

After 18 months, the hemoglobin concentration remained at 7 g/dl and the reticulocyte count was 13×10^9/l (0.5%). Bone marrow aspiration revealed pure red cell aplasia. Bone marrow and serum PCR for PVB 19 were highly positive and the patient was positive for anti-PVB 19 IgM and IgG. Serum creatinine concentration was stable at 140 μmol/l. Cyclosporine trough levels ranged between 150 and 200 ng/mL.

A second course of IVIG was administered (0.5 g/kg/day of body weight for 2 days per month for 4 months). The rHuEPO therapy was discontinued. One month later, the hemoglobin concentration reached 11 g/dl and the reticulocyte count rose to 58×10^9/l. After the last course of IVIG, the serum hemoglobin concentration was 13.5 g/dl and the reticulocyte count was 126×10^9/l (3%). The serum PVB 19 PCR had dropped to 10^6 copies/ml. At the last follow-up visit, 32 months after transplantation, the serum hemoglobin concentration was 13 g/dl. Serum PCR and anti-PVB 19 IgM became negative, although IgG remained positive (Fig. 1).

Discussion

Parvovirus B19-related chronic pure red cell aplasia has been observed only in patients with hereditary or acquired immunodeficiency syndromes, including immunosuppressive therapy chemotherapy and AIDS [6]. The main mechanism facilitating this viral infection is a defect of humoral immunity, particularly an inefficient isotopic IgM/IgG switch. The lack of circulating anti-PVB 19 IgG does not allow the complete clearance of the virus and results in chronic infection. This condition has also been reported in organ transplant recipients [7,8]. In most cases, viral infection, complicating renal transplantation is a primary infection resulting from the transmission of the virus through the grafted organ, red blood cell transfusions or contamination with respiratory secretions [7]. In our case, the seroconversion observed in the organ recipient is suggestive of primary infection. As the patient was not transfused until anemia occurred, PVB 19 may have been transmitted through the kidney graft, possibly hosted in endothelial cells [9].

In transplanted patients, the eradication of infection may be long, and require both the reduction of immunosuppression and the administration of high-dose IVIG [7,8,10,11]. However, infection has also been reported to resolve spontaneously [1], or following the reduction of immunosuppression without immunoglobulins [12,13]. In our case, the persistence of PVB 19 infection was probably favored by the maintenance of the immunosuppressive regimen. Indeed, tacrolimus is a potent inhibitor of the helper T-cell response that is required to develop the anti-viral T cytotoxic and humoral responses [12,14], and MMF has been demonstrated to reduce the humoral response by directly targeting B cells [15,16]. However, discontinuation of MMF and the switch from tacrolimus to cyclosporine were not sufficient to treat PVB 19 infection as suggested in previous reports [12,13].

Many clinical reports mention that IVIG are efficient in achieving a hematological response, and in some cases a virological response. Various therapeutic regimens have been reported with a favorable outcome. Cumulative doses usually range from 35 to 250 g [10]. Our patient was treated both times with doses within these ranges. Indeed, the cumulative dose after the first IVIG therapy was 120 g in four 30 g courses. During the second

treatment with IVIG, the cumulative dose was 240 g in four 60 g courses. However, we cannot rule out that the failure of the first step of IVIG therapy was due to the injection schedule. Indeed, the dose of IVIG per course may have been too weak (30 g) and the delay between injections may have been too long (4 weeks).

Nevertheless, failure of initial course of IVIG to treat PVB 19 infection may also be due to the concomitant use of rHuEPO. Indeed, high doses of rHuEPO (300 U/kg/week) were given, because this patient had nonregenerative anemia. In our case, by stimulating progenitor cell replication, rHuEPO may also have stimulated viral replication and provided new target cells. Indeed, rHuEPO has been demonstrated to facilitate PVB 19 replication and to delay recovery from anemia. *In vivo* and *in vitro* studies have shown that human erythroid progenitor cells derived from bone marrow or fetal liver are the targets of virus infection [17,18]. PVB 19 must bind to the erythrocyte P antigen to enter the target cell. Cells expressing this surface antigen include erythroblasts, erythrocytes, megakaryocytes, endothelial cells, hepatocytes and cardiomyocytes, but viral replication and production can occur only in erythroid progenitor cells [burst forming unit-erythroid (BFU-E) and colony forming unit-erythroid (CFU-E)]. Indeed, PVB 19 is a single-strand DNA capsid virus, devoid of DNA polymerase. Consequently, this virus can replicate only in dividing cells, in which DNA polymerase is activated. Stimulation by rHuEPO and interleukin 3 of erythroid progenitor cells generated *in vitro* from peripheral human blood, and subsequently infected by human PVB 19 result in a high level of viral DNA replication, production of viral proteins and production of infectious virus [18]. Erythroblasts derived from the more mature progenitor cells CFU-E are more sensitive than BFU-E *in vitro* to PVB 19 infection. PVB 19-induced cell lysis is predominant at stages of maturation that are dependent only on EPO and increases with EPO concentration [5].

However, the role of EPO in the promotion of PVB 19 viral replication is controversial in some *in vitro* and clinical studies. Leruez *et al.* [19] aimed to evaluate the presence and localization of PVB 19 nucleic acids and proteins in infected UT7/EPO cells. As bone marrow is difficult to obtain, the authors have used an erythropoietin-dependent leukemic cell line, UT7. The UT7 cell line is pluripotent and demonstrates an erythroid differentiation on induction by EPO (UT7/EPO cells). After infection with PVB 19, no viral capsid proteins were detected in UT7/EPO cells. Approximately 20% of the infected UT7/EPO cells demonstrated viral transcripts of non-structural genes, whereas only 5% of these cells expressed transcripts of structural genes. The authors concluded that UT7/EPO cell line is not permissive to B19 replication but does support viral transcription. This cell line constitutes an experimental example, in which EPO does not stimulate viral replication.

Controversial clinical reports on the role of EPO have also been published. Wicki *et al.* [20] reported the cases of two solid-organ transplant recipients (one lung and one heart) with PVB 19-induced red cell aplasia. In both cases, resolution of anemia was rapidly reached (within 2 weeks) following a 5-day course of IVIG (0.4 g/kg/day) associated with rHuEPO (10 000 U s.c. three times a week for 3 weeks). The authors hypothesize that time to recovery may be shortened by using rHuEPO. Clearly in these cases, rHuEPO does not seem to have played a negative role.

In conclusion, our case suggests that the use of rHuEPO in transplant patients with PVB 19-induced anemia may enhance viral replication and impede response to classical treatments. Despite controversial results in the literature, we think that before using rHuEPO in transplant patients, a parvovirus infection should be ruled out. As the secretion of endogenous EPO is always restored in renal transplant patients, except in cases of severe allograft dysfunction, the use of rHuEPO could be limited to anemia not associated with PVB 19 infection. In the case of PVB 19 infection, we suggest that rHuEPO should be avoided and that immunosuppressive therapy be reduced. If these strategies fail, a treatment with IVIG has to be used to achieve a clinical response. However, further studies to precise the role of EPO on viral replication *in vitro* and *in vivo* are necessary.

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