Severe Prolonged Red Blood Cell Aplasia and Thrombocytopenia Induced by Parvovirus B19 Infection in a Patient with Sarcoidosis

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We describe an acromegalic patient who developed a parvovirus B19 (PVB19) infection concomitantly with sarcoidosis, which was complicated by chronic red blood cell aplasia and severe thrombocytopenia despite concomitant sarcoidosis, which was complicated by chronic RBC aplasia and severe thrombocytopenia despite conserved humoral immunity.

Materials and methods. ELISAs were used to detect serum IgM and IgG directed against PVB19 on the basis of capsid antigens (Biotrin SARL) [11]. PVB19 DNA was extracted from 200 mm² of peripheral blood on silica columns (QIamp DNA Blood Mini; Qiagen) and amplified by PCR with use of the following primers: 5′-GTGCTTACCTGTCGTGGATTGC-3′ and 5′-GCTAACCTGCCCCAGGCTTGT-3′. This PCR amplification generated a 402-bp fragment, which was identified after agarose gel electrophoresis, ethidium bromide staining, and observation under ultraviolet light.

Case report. A 36-year-old woman was referred to Haut-Lévêque Hospital (Bordeaux, France) for severe acute anemia. Two months earlier, she had consulted an endocrinologist, complaining of a 10-year history of acral enlargement, headaches, and menstrual abnormalities. Basal growth hormone and prolactin levels were elevated, and MRI scans of the sella turcica revealed intrasellar and suprasellar lesions that were diagnosed as a pituitary adenoma. The findings of a hemogram obtained at that time were normal, with a hemoglobin level of 13.5 g/dL, a WBC count of 5 × 10⁹ cells/L, and a platelet count of 94.4 × 10⁹ platelets/L (figure 1A).

Surgical transphenoidal excision of the lesion was scheduled, but the patient developed a fever (temperature, 38.5°C) and dyspnea without cough or sputum production. Physical examination performed at the time of presentation (day 1) detected splenomegaly without hepatomegaly. Blood analyses revealed the following values: WBC count, 5 × 10⁹ cells/L (49% granulocytes and 45% lymphocytes); RBC count, 10¹² cells/L; hemoglobin, 3.6 g/dL; hematocrit, 11%; mean corpuscular volume, 107 μm³ (normal range, 85–95 μm³); reticulocyte count, 20 × 10⁹ cells/L; platelet count, 49 × 10⁹ platelets/L; negative direct Coombs’ test result; normal haptoglobin level; aspartate aminotransferase, 50 U/L (normal range, 7–40 U/L); and alanine aminotransferase, 145 U/L (normal range, 5–50 U/L). Serum vitamin B₁₂ and folate levels were normal, but the ferritin level was high (1384 ng/mL; normal range, 21–247 ng/mL). The erythrocyte sedimentation rate was 150 mm/hour, the C-reactive protein level was 107 mg/L (normal, <10 mg/L), and polyclonal hypergammaglobulinemia was present. Absolute

Received 17 June 2002; accepted 14 October 2002; electronically published 6 January 2003.

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Clinical Infectious Diseases 2003;36:229–33

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1058-4838/2003/3602-0017$15.00
Figure 1. Findings of examinations of blood samples obtained during follow-up. A, Hemoglobin and platelet levels. B, Total CD3+ and CD3+CD4+CD8+ lymphocyte levels. IVIG, intravenous immunoglobulin.

CD4+ and CD8+ T lymphocyte counts were 0.730 × 10^7 cells/L and 1.223 × 10^7 cells/L, respectively (normal ranges, 0.7–1.1 × 10^7 cells/L and 0.6–0.9 × 10^7 cells/L, respectively). The CD3+CD4−CD8− lymphocyte subset level was abnormally high (0.258 × 10^7 cells/L, 8% of the total CD3+ lymphocyte count) and expressed the γδT cell receptor.

One-half of the CD3+ T lymphocytes (1.2 × 10^7 cells/L) expressed human leukocyte antigen DR at their surface, suggesting that they were activated, and most of them had the CD3+CD4−CD8− phenotype. Examination of a bone marrow aspirate obtained on day 2 showed hypocellularity and erythroid aplasia without characteristic giant pronormoblasts. Megakaryocytes were rare, but granulocytic differentiation was normal. Drug-induced aplasia was ruled out because the patient denied having taken any drugs. 51Cr labeling of autologous RBCs indicated that their shortened life span (5 days) was associated with splenic sequestration. The results of serological tests to assess autoimmunity and virus exposure were negative, except for IgG to cytomegalovirus and Epstein-Barr virus. Human PVB19 DNA and anti-PVB19 IgM were found in a serum sample obtained on day 1. CT scans of the thorax and abdomen revealed marked splenomegaly at 22 cm and bilateral hilar and multiple right-side paratracheal lymphadenopathies. Histological examination of thoracic lymph node tissue samples revealed areas of fibrosis and multiple nonnecrotizing granulomas containing giant cells and epithelioid cells. No acid-fast bacilli or foreign bodies were seen.

PVB19-induced RBC aplasia and thrombocytopenia was diagnosed in this patient, who had sarcoidosis. She received repeated RBC and platelet transfusions as well as IVIG (400 mg/kg q.d. on days 15–20), and oral prednisolone (1 mg/kg q.d.) was initiated 8 weeks after admission to the hospital. One month later (day 84), circulating anti-PVB19 IgG and IgM were detected, although PVB19 DNA had disappeared. The number of activated CD3+CD4+CD8+ lymphocytes decreased, as assessed by the diminished DR surface expression, but the in-
creased CD3⁺CD4⁺CD8⁻ subset bearing the γδ T cell receptor persisted (figure 1B). CT scan of the chest revealed regression of the adenopathy, which completely disappeared 3 months after the onset of corticotherapy. However, hematopoiesis did not recur while the patient was receiving corticosteroids, and the values noted on blood analysis worsened (figure 1A), as follows: WBC count, cells/L (29% granulocytes and 95.3% lymphocytes); RBC count, cells/L; hemoglobin, 122.5 g/dL; reticulocyte count, cells/L; and platelet count, 920 x 10⁶ platelets/L. The patient needed regular RBC and platelet transfusions, and the results of blood tests did not improve after she underwent splenectomy (day 190). Examination of a sample of splenic tissue showed congested sinusoids in the red pulp containing several hemosiderin-filled macrophages and a mildly elevated lymphocyte (CD4⁺) count. There was no evidence of a hemolytic process: hemoglobin electrophoresis findings, osmotic fragility findings, serum haptoglobin levels, and glucose-6-phosphate dehydrogenase levels were normal. Marchiafava-Micheli disease was ruled out. Although the patient underwent splenectomy, the bicipotemia worsened (hemoglobin level, 5 g/dL; platelet count, 3 x 10⁷ platelets/L), and the patient required more RBC and platelet transfusions. No alloimmunization was detected, and transfusions were effective. An additional bone marrow biopsy revealed hypocellularity without erythroid precursors and megakaryocytes; the granular lineage was normal, and there was no dysplasia. Combined antilymphocyte globulins and cyclosporine were then prescribed, but the patient died of a cerebral hemorrhage (day 310).

The potential contributions of IL-2, IL-10, and IFN-γ to our patient’s clinical features were investigated by examining spontaneous and mitogen-induced syntheses of these cytokines by PBMCs by means of the previously described whole-blood assay, which appears to better preserve the natural environment and constitutes an appropriate milieu in which to study in vitro cytokine production [12, 13]. Basal and mitogen-induced cytokine levels were measured in whole blood samples with use of ELISA after the splenectomy was performed (table 1). The IFN-γ level was dramatically increased in our patient after mitogen stimulation, whereas IL-2 and IL-10 levels were comparable to those of control subjects.

**Discussion.** The presence of PVB19-specific IgM, followed by the presence of IgG antibodies and DNA, clearly indicates an acute PVB19 infection. The diagnosis of sarcoidosis was also well established on the basis of typical radiological findings, well-defined epithelioid granulomas with giant cells, and complete regression of lymphadenopathy after the administration of corticotherapy. As suggested by the isotopic examination, which showed a shortened RBC life span due to splenic sequestration, we hypothesize that the enlarged spleen led to hemolysis with reticulocytosis favoring PVB19 infection of erythroid precursors.

PVB19 is a single-stranded DNA virus that produces a wide spectrum of clinical manifestations, but erythroid precursor destruction constitutes the main underlying cause of its associated morbidity and mortality. Virus clearance and consequent recovery of erythropoiesis are contingent on an adequate antibody response to the virus; this occurs within 2–3 weeks in most healthy persons. Patients who are unable to mount such a humoral response (e.g., those with chronic anemia due to
persistent PVB19 infection related to congenital [8] or acquired immunodeficiency [9]), however, do not clear the virus. Our case is remarkable for the severe, chronic RBC aplasia associated with amegakaryocytopenia, despite virus disappearance from serum and the production of high levels of specific polyclonal antibodies to PVB19, attesting to normal humoral immunity sustained by normal secretion of the type 2 cytokine IL-10. Moreover, the disappearance of viral DNA from the blood 2 months after the onset of disease was confirmed by repeated PCR. This suggests that PVB19 is not directly responsible for the continuing marrow aplasia.

Sarcoidosis is a multisystem disease of unknown etiology that is characterized by noncaseating granulomas and the accumulation of T cells and mononuclear phagocytes at sites of inflammation. The lungs are the most frequently affected organs, with increased percentages of activated CD4+ T cells present in the lung interstitium, and recent evidence suggests that T cells at inflammation sites are oligoclonal, which is consistent with a conventional antigen-driven process. There have been reports of polarization of the T cell response in pulmonary sarcoidosis, with increased expression of the type 1 cytokines IL-2 and IFN-γ and little or no detectable expression of the type 2 cytokines IL-4 and IL-5 in the sarcoid lung [14–16]. Although these results suggest compartmentalized deviation to type 1 cytokine expression at sarcoidosis disease sites, elevated levels of circulating IFN-γ have been reported in some patients [17]. Mitogen stimulation of our patient’s PBMCs induced oversecretion of IFN-γ, which may represent a simulation of sarcoidosis. Because hematopoietic suppression by IFN-γ has been reported [18], a possible mechanism underlying our patient’s reticuloendothelial and thrombocytopenia could involve IFN-γ. In addition, peripheral blood lymphocytes obtained from patients with aplastic anemia are known to spontaneously secrete or oversynthesize IFN-γ after lectin stimulation [19,20].

The constant presence of high numbers of circulating γδ T cells in our patient is also intriguing, but is probably due to sarcoidosis, as has been observed by Balbi et al. [21]. Expansion of circulating γδ T cells in patients with active sarcoidosis has been closely correlated with defective cellular immunity [22]. The persistence of γδ T cells throughout the course of aplasia despite the cure of the sarcoidosis suggests a possible link between the 2 factors, in accordance with the findings of Hara et al. [23], who reported the participation of γδ T cells in the pathogenesis of pure RBC aplasia in a patient with type 1 autoimmune polyendocrinopathy. Unfortunately, we did not isolate γδ T cells, test their IFN-γ secretion, or perform a hematopoietic progenitor cell assay.

In conclusion, the sequence of events—showing that the viremia cleared coincident with the onset of humoral immunity—suggests that PVB19 infection is not directly responsible for continuing marrow aplasia. Our data concerning IFN-γ secretion reflect a post–viral infection, immune-mediated response in the presence of an underlying disorder (sarcoidosis) that resulted in abnormal cytokine release.

References

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