The Use of Monoclonal Antibody R92F6 and Polymerase Chain Reaction to Confirm the Presence of Parvovirus B19 in Bone Marrow Specimens of Patients With Acquired Immunodeficiency Syndrome

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Background. Parvovirus B19 infection is a cause of chronic anemia and red cell aplasia in patients with acquired immunodeficiency syndrome (AIDS) and in other immunocompromised hosts. Anemia in AIDS patients has a multifactorial etiology, with parvovirus B19 infection being an infrequent but nevertheless treatable cause. Therapy with intravenous immune globulin can result in rapid improvement of parvovirus-induced anemia. This treatment is expensive, therefore accurate and rapid confirmation of parvovirus infection is important in providing appropriate and cost-effective therapy.

Methods. Bone marrow samples from 2 AIDS patients with severe anemia and reticulocytopenia were studied. Bone marrow morphology and serologic studies were evaluated for parvovirus B19 infection. An immunohistochemical method using a monoclonal antibody, R92F6, to B19 capsid proteins was utilized on decalciﬁed, B5-ﬁxed, parafﬁn-embedded bone marrow biopsies. Bone marrow aspirate cells were examined by electron microscopy for evidence of viral particles. In addition, polymerase chain reaction (PCR) studies using a nested PCR assay to the parvovirus B19 viral genome were performed in a case for which fresh cells were available.

Results. Bone marrow ﬁndings included marked erythroid hypoplasia with characteristic giant pronormoblasts and intranuclear inclusions. Serologic studies were negative in one case, while the second case showed positive parvovirus B19 immunoglobulin M antibody. Immunohistochemical studies for parvovirus B19 were positive in both cases. The presence of intranuclear virions was demonstrated by electron microscopy and was conﬁrmed by PCR analysis. Both patients were treated with intravenous immune globulin, and subsequent improvement was noted.

Conclusions. Both immunohistochemistry and PCR studies on bone marrow specimens from AIDS patients with anemia are rapid and sensitive methods for the conﬁrmation of parvovirus B19 infection. They are valuable tools, particularly when serologic studies are negative. When PCR is not available, immunohistochemical methods can be useful. The rapid conﬁrmation of parvovirus B19 infection will allow for early and cost-effective therapy.

( Arch Pathol Lab Med. 1999;123:768±773)
tion, or polymerase chain reaction (PCR) techniques. A sensitive nested PCR technique has been developed.

Immunohistochemical methods using the monoclonal antibody R92F6 have also made the confirmation of parvovirus B19 infection possible on routinely processed tissues.

We present 2 cases involving patients with acquired immunodeficiency syndrome (AIDS) and severe anemia and reticulocytopenia. We also describe bone marrow morphologic findings and demonstrate the usefulness of immunohistochemistry and PCR studies on bone marrow specimens to confirm the presence of suspected parvovirus B19 infection.

MATERIALS AND METHODS

Clinical Specimens

Medical records were reviewed for clinical history, and bone marrow aspirates, clots, and biopsies were studied. Bone marrow aspirates were air-dried and stained with Wright-Giemsa. Bone marrow clots and biopsies were fixed in B5, and biopsies were decalcified (Decal-Stat, Decal Chemical Corporation, Congers, NY). The specimens were routinely processed and embedded in paraffin. Five-micrometer sections were cut and stained with hematoxylin-eosin.

Immunohistochemistry

Immunoperoxidase staining for parvovirus B19 VP1 and VP2 capsid proteins was performed on 5-μm paraffin sections prepared from the bone marrow biopsy and clot specimens. Following routine deparaffinization, rehydration, and heat-induced epitope retrieval, tissue sections were incubated overnight with a 1: 500 dilution of the monoclonal antibody R92F6 (Novocastra Laboratories Ltd./Vector Laboratories Inc, Burlingame, Calif). The detection system consisted of biotinylated Fab′/b, rabbit anti-mouse immunoglobulin (1:500; Dako Corporation, Carpenteria, Calif) and horseradish peroxidase-conjugated streptavidin (1: 1000; Jackson ImmunoResearch Laboratories, Inc, West Grove, Pa). Sections were incubated with the chromogen aminoethylcarbazole (Sigma Chemical Company, St Louis, Mo), counterstained with hematoxylin, and coated with Crystal Mount (Biomeda Corp, Foster City, Calif) prior to coverslipping. Appropriate positive and negative controls were run in parallel.

Polymerase Chain Reaction Analysis

Polymerase chain reaction was performed on DNA extracted from bone marrow cells (case 1) using a nonorganic extraction kit (Puregene, Gentra Systems Inc, Minneapolis, Minn). A nested PCR technique was used with a first-round PCR using the outer primer pair Parpat-1 (CTTTAGGTATAGCCAACTGG) and Parpat-3AS (ACACTGAGTTTACTAGTGGG). Approximately 1 μg of genomic DNA was incubated in a total reaction volume of 50 μL containing 200 μmol/L each deoxyribonucleoside triphosphates (dNTP), 1.5 μmol/L Tris-HCl, pH 8.3, 50 mmol/L KCl, 1.5 mmol/L MgCl₂, 0.001% (wt/vol) gelatin (Perkin-Elmer), and 150 ng each of forward and reverse primers. The PCR reaction was performed in a Perkin-Elmer (Perkin-Elmer, Norwalk, Conn), reaction buffer (10 mmol/L Tris-HCl, pH 8.3, 50 mmol/L KCl, 1.5 mmol/L MgCl₂, 0.001% [wt/vol] gelatin) (Perkin-Elmer), and 150 ng each of forward and reverse primers. The PCR reaction was performed in a Perkin-Elmer (Perkin Elmer 2400) thermal cycler. The PCR protocol consisted of initial denaturation at 94°C for 5 minutes, followed by 30 cycles, each comprising 30 seconds of denaturation at 94°C, 30 seconds of annealing at 55°C, and 30 seconds of extension at 72°C. Following the first-round PCR, 5 μL of the PCR product was used for the second-round PCR with the inner primer pair B19-1 (CAGAACCATGTGGACTGAGG) and B19-2 (CTTATAATGCCTCCTFCAGG) in the same way as described for the outer primers. The amplified DNA was visualized following separation by gel electrophoresis in 10% polyacrylamide gels and subsequent staining with ethidium bromide.

Electron Microscopy

Bone marrow aspirate cells for electron microscopy were prepared by established techniques. The bone marrow aspirate buffy coat was fixed in 3% glutaraldehyde, and then pelleted and embedded in 2% agar. The hardened agar was then cut into 1-mm cubes and processed routinely. Thin sections were examined and photographed on a Zeiss EM10CR electron microscope.

REPORT OF CASES

Case 1

A 33-year-old man with a 10-year history of human immunodeficiency virus (HIV) infection and advanced-stage disease (CD4+ T-cell count of 6/mm³) presented with anorexia, easy fatigability, and a several-week history of transfusion-dependent anemia. Three weeks earlier, the patient had a hematocrit of 0.13 and required 4 units of packed red blood cells. Laboratory evaluation on admission showed a white blood cell count of 4.3 × 10⁹/L with 65% granulocytes, 20% lymphocytes, 10% monocytes, 3% eosinophils, and 2% basophils; hemoglobin, 81 g/L; hematocrit, 0.24; mean corpuscular volume, 90.8 fL; and platelets, 613 × 10⁹/L. The patient's reticulocyte count percentage was 0.6%, and his absolute reticulocyte count was 16.9 × 10⁹/L.

A bone marrow aspirate and biopsy were performed. The aspirate, clot, and biopsy showed hypercellular bone marrow with granulocytic and megakaryocytic hyperplasia and marked erythroid hypoplasia with little maturation beyond the pronormoblast stage. Occasional giant pronormoblasts were identified on the aspirate smear (Figure 1). Erythroid precursors with intranuclear inclusions and occasional giant pronormoblasts were identified in the clot and biopsy sections (Figure 2). The findings were suggestive of parvovirus B19 infection. Immunoperoxidase studies with monoclonal antibody R92F6 performed on the biopsy and clot sections showed predominantly intranuclear reactivity in scattered cells, confirming the presence of parvovirus B19 infection (Figure 3). Polymerase chain reaction studies showed an amplified DNA fragment, also consistent with the presence of parvovirus B19 infection (Figure 4). Serologic titers for IgM and IgG antibodies to parvovirus B19 antigens were negative. Immunoperoxidase studies for mycobacteria, histoplasmosis, and cytomegalovirus were also negative. Parvovirus organisms were also subsequently demonstrated by electron microscopy (Figure 5). Eighteen- to 26-nm nucleocapsids were observed intimately associated with nucleolar material in several erythroid precursor cells.

Therapy with IVIG was initiated at 20 g/d for 5 days. After the fifth day of treatment, the patient's reticulocyte count percentage increased to 6.1%, and the absolute reticulocyte count increased to 168.4 × 10⁹/L. Two weeks later, the patient's follow-up hematocrit was stable at 0.35.

Case 2

A 39-year-old woman presented with painful genital lesions and generalized fatigue. She had been diagnosed as HIV-positive 1 year previously, at which time she presented with a CD4+ T-cell count of 1/mm³. Laboratory evaluation on admission showed a white blood cell count of 2.6 × 10⁹/L with 1% metamyelocytes, 10% bands, 46% neutrophils, 9% monocytes, 6% eosinophils, and 2% basophils; hemoglobin, 62 g/L; hematocrit, 0.18; mean corpuscular volume, 87.9 fL; and platelets, 251 × 10⁹/L. The patient's reticulocyte count percentage was 1.2%, and her absolute reticulocyte count was 24.7 × 10⁹/L. She received 2 units of packed red blood cells and subsequently showed no response to treatment with Epogen.

A bone marrow aspirate and biopsy were performed. The aspirate material contained no marrow particles. The bone marrow clot and biopsy showed a normocellular marrow with marked erythroid hypoplasia. Occasional giant pronormoblasts and erythroid cells with intranuclear inclusions were identified, suggestive of parvovirus B19 infection. Immunoperoxidase studies showed strong reactivity to the monoclonal antibody R92F6, con-
Figure 1. Case 1. Giant pronormoblast (arrowhead) in bone marrow aspirate smear (Wright-Giemsa, original magnification ×500).

Figure 2. Case 1. A, Giant pronormoblast (arrowhead) with intranuclear inclusion. Bone marrow biopsy (hematoxylin-eosin, original magnification ×1000). B, Intranuclear inclusions (arrowheads) in erythroid cells. Bone marrow biopsy (hematoxylin-eosin, original magnification ×1000).

Figure 3. Case 1. Immunoreactivity to monoclonal antibody R92F6. Bone marrow biopsy (original magnification ×1000).

Figure 4. Case 1. Gel electrophoresis of nested polymerase chain reaction products showing amplified parvovirus DNA. Lane M, molecular size markers; lane 1, blank control; lane 2, negative control; lane 3, positive control; lanes 4 and 5, positive case 1; and lanes 6 and 7, negative patient specimen.
Figure 5. Case 1. Electron micrograph of parvovirus-infected cell with intranuclear clusters of virus particles (arrows) (original magnification ×11500). Inset, High-power view of parvovirus particle. Bar = 25 nm.

Figure 6. Case 2. Immunoreactivity to monoclonal antibody R92F6. Bone marrow biopsy (original magnification ×1000).
firming the presence of parvovirus B19 infection (Figure 6). Parvovirus B19 serological studies were positive for IgM and negative for IgG antibodies. Immunoperoxidase studies for cytomegalovirus, herpes simplex virus, varicella virus, and mycobacteria were negative. Polymerase chain reaction analysis was not performed on this case owing to a lack of fresh cells or non-B5-fixed tissue.

Therapy with IVIG was initiated at 22 g/d and was continued for 7 days. Although the patient’s hematocrit initially decreased, 5 days following completion of IVIG, the reticulocyte count percentage increased to 5.1%, and the absolute reticulocyte count increased to 162.7 x 10^6/L. The patient’s hematocrit subsequently increased.

COMMENT

Anemia is a common problem in AIDS patients and can result from a variety of causes, including infections, malignancies, nutritional factors, immunologic impairment, and medications. Parvovirus B19 is one of the multitude of infectious agents that lead to significant morbidity in AIDS patients. Recent studies indicate that parvovirus B19 infection is relatively uncommon in HIV-infected and AIDS patients. In a study by Chernak et al.,18 1 of 105 HIV-infected individuals and 1 of 22 HIV-infected patients with anemia showed evidence of parvovirus infection by PCR studies on serum. Liu et al.17 found evidence of parvovirus infection in 7 of 81 bone marrow specimens from AIDS patients by in situ hybridization techniques and concluded that a small but significant number of bone marrow specimens showed evidence of parvovirus infection. Because effective IVIG therapy is available, when parvovirus infection is suspected, rapid and accurate diagnostic methods are important. The avoidance of unnecessary red blood cell transfusions and the prevention of viral transmission to other immunocompromised hosts and pregnant women are other important considerations.

Both our cases presented with anemia and reticulocytopenia, and bone marrow findings were suggestive of parvovirus infection. Red cell hypoplasia, characteristic giant pronormoblasts, and intranuclear inclusions were noted. Giant pronormoblasts and viral inclusions are, however, not always identified. Both PCR and immunohistochemical studies can provide rapid confirmation of suspected parvovirus infection. The usefulness of PCR techniques in the diagnosis of parvovirus infection has been reported previously; however, another recent option for diagnosis is the use of immunohistochemical techniques.

The parvovirus genome encodes for a nonstructural protein and 2 capsid proteins (VP1 and VP2). The monoclonal antibody R92F6, produced by O’Neill and Coyle, is specific for the 2 viral capsid proteins. Morey et al.20 reported the use of this antibody in 1992 for labeling infected cells in paraffin-embedded tissues from 19 cases of parvovirus-related fetal hydrops and in a bone marrow specimen from a child with congenital immunodeficiency. Other reports have also demonstrated the usefulness of immunohistochemistry in detecting parvovirus in intrauterine fetal demise.26,27 Jordan and Penchansky16 examined 15 bone marrow specimens of pediatric patients with acute anemia and found 4 cases with evidence of parvovirus infection. They found good correlation between morphologic, immunohistochemical (using B19-specific monoclonal antibody, Chemicon, on bone marrow aspirate smears), and PCR studies.16

In a recent large study by Liu et al.,17 81 paraffin-embedded bone marrow core biopsies and clots from AIDS patients were studied using the monoclonal antibody R92F6 and in situ hybridization methods. Seven of 81 cases were positive for B19 parvovirus DNA by in situ hybridization techniques; however, all cases were negative with R92F6. In contrast, Morey et al.20 did find correlation between previously performed in situ hybridization results and their immunohistochemical findings. They noted the strength of immunoreactivity decreased with prolonged fixation and autolysis; however, detection with R92F6 was as sensitive as in situ hybridization in specimens fixed for less than 24 hours.

With respect to immunoreactivity, Morey et al.20 noted cytoplasmic staining in some cells, while in others strong nuclear reactivity was noted. Our cases also showed strongest staining in inclusion-containing cells. Although in some studies giant pronormoblasts and intranuclear inclusions were identified, Liu et al.17 found only 3 of 8 parvovirus-infected bone marrow specimens had rare inclusions, and in their study, in situ hybridization was more sensitive than morphologic diagnosis. Morey et al.20 found 9 of 19 cases of fetal hydrops showed no evidence of inclusions, but the cases did show a small number of cells to be positive with R92F6 antibody or in situ hybridization. They concluded that additional investigations were necessary, or low-grade or resolving parvovirus infection would be missed.

Frickhofen et al.11 reported the effectiveness of IVIG therapy in 6 HIV-positive patients with parvovirus infection. In AIDS patients, it is noted that there is an intense parvoviremia, and IVIG treatment appears to reduce but not eliminate the virus; consequently, relapse is not uncommon.

In the study by Frickhofen et al, 3 patients relapsed and were successfully retreated with IVIG. It is noted, however, that AIDS patients with other opportunistic infections, such as Mycobacterium avium, may be difficult to treat.

In summary, although parvovirus B19 infection is an uncommon cause of anemia in AIDS patients, it is readily treatable. Immunohistochemical studies with R92F6 and PCR studies are both useful techniques in the diagnosis of suspected parvovirus infection. When PCR studies are not available, immunohistochemical studies can be performed on routinely processed bone marrow clots and biopsies. Rapid and cost-effective IVIG can thus be provided to the appropriate patients.

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