Human Parvovirus B19-Induced Epidemic Acute Red Cell Aplasia in Patients With Hereditary Hemolytic Anemia

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From March to August 1984, 26 patients with hereditary hemolytic anemia in northeastern Ohio developed acute, profound red cell aplasia. The patients included 14 males and 12 females 2 to 23 years old, with sickle cell anemia (20 cases), hemoglobin SC-disease (4 cases), sickle-beta-thalassemia (1 case), or hereditary spherocytosis (1 case). All had an acute onset of severe reticulocytopenia and anemia and prodromal symptoms of illness including fever, abdominal symptoms, headache, and arthralgias. Twenty-two received transfusions. Reticulocytosis occurred spontaneously within 2 to 14 days of presentation. In five acute-phase sera, \(^{10}\) to \(10^{12}\) viral particles/mL were detected by electron microscopy. Human parvovirus B19 DNA was demonstrated in high concentration by hybridization in the same five acute-phase sera and in low concentration in sera of eight additional patients. The five highly viremic sera inhibited erythroid colony formation in vitro. B19-specific IgM was detected in sera of 24/26 patients, and B19-specific IgG in 21 of 22 patients tested. Our results indicate that human parvovirus B19 was the etiologic agent in this large epidemic of life-threatening acute red cell aplasia in patients with hereditary hemolytic anemia.

Between March 13 and August 23, 1984, all known patients with hereditary hemolytic anemia and acute red cell aplasia admitted to hospitals in northeastern Ohio were prospectively enrolled in the study. During the last 4 months of 1984, no new cases appeared. Nineteen patients were admitted to Rainbow Babies and Childrens Hospital, Case Western Reserve University, Cleveland; one to Lakeside Hospital, the adult-care facility of the University Hospitals in Cleveland; one to St. Luke’s Hospital, Cleveland; two to Kaiser-Permanente, Cleveland Heights and Parma; two to Akron Children’s Hospital, Akron; and one to St. Joseph’s Hospital, Lorain.

Case definition. A case of aplastic crisis was defined as a patient with underlying hereditary hemolytic anemia, who developed red cell aplasia of less than 2 weeks of duration, with a fall in the reticulocyte count to less than 50% of the patient’s mean baseline value. The mean baseline reticulocyte count was the average value obtained from well-clinic visit records. Although most patients developed severe anemia requiring transfusion, hemoglobin or hematocrit values were not used as criteria for case definition.

Methods. A detailed history was taken, especially regarding preceding symptoms of illness. Additional information was obtained in most cases by telephone interview of the patient’s mother or another adult family member. Physical examination was performed at the time of hospital admission and repeated daily until discharge. Peripheral blood counts, including hematocrit (Hct), hemoglobin (Hgb), reticulocyte count, platelet count, white blood cell count (WBC), and differential were measured during the hospitalization and the follow-up visits. The baseline levels of Hct and reticulocyte count were defined as the average of well-clinic visit records.

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preferably in the previous 2 years, or when unavailable, from follow-up visits more than 2 months after hospital discharge. Venous blood was drawn within the acute and/or convalescent phase for virologic and serologic studies. The serum was stored at −70 °C until analyzed.

**Laboratory methods.** Acute-phase sera were evaluated for human parvovirus particles by negative-stain electron microscopy with uranyl acetate stain. B19 DNA was estimated in the sera by a modification of the DNA dot hybridization method. In the hybridization procedure, aliquots corresponding to 0.5 μL of serum were digested with 100 μg/mL proteinase K for 30 minutes at 60 °C; an equal volume of 0.6N NaOH was then added, and the sample was hydrolyzed at 60 °C for another 30 minutes. An equal volume of 2 mol/L ammonium acetate (pH 7.0) was then added, and the sample was applied to pre-wet nitrocellulose filters with a slot-blotter apparatus (Schleicher and Schuell, Keene, NH). Slot blots were probed with high specific activity 32P-labeled RNA, synthesized in vitro from a full-length copy of B19 DNA inserted in the SP65 riboprobe vector. Probe synthesis and hybridization were performed as described by Zinn et al and B19 DNA concentrations estimated by densitometric comparison with standards constructed by dilution, in negative serum, of purified 110S B19 virions, with extinction coefficients derived for the minute virus of mice, another parvovirus. The limit of detection was 0.30 ng/mL of serum. The viral DNA from acute-phase aplastic crisis sera was compared with prototype B19 DNA on alkaline agarose gels. The virions from one case were purified and compared with other parvoviruses.

The ability of viremic sera to inhibit erythroid colony formation in vitro was determined using normal bone marrow cells cultured in methylcellulose, by methods previously described. Under the conditions of testing (dilutions of sera of 10-1 or greater, the absence of added complement, and adsorption at 4 °C) sera from normal individuals or those who have received multiple blood transfusions do not significantly inhibit erythroid or myeloid colony formation.

All sera were evaluated for B19-specific IgM and IgG antibodies in a capture enzyme-linked immunosorbent assay (ELISA) patterned after the radioimmunooassay method. In the ELISA, goat antihuman IgG or IgM were used as capture antibody (Tago Laboratories, Burlingame, Calif) followed by the human serum sample, diluted 1:100, then B19 antigen from the acute-phase serum of an aplastic crisis patient, diluted 1:500, then a mouse monoclonal antibody (162-2B) against B19, and finally goat antimouse IgG H- and L-chain conjugated with peroxidase (Kirkegaard and Perry Laboratories, Gaithersburg, Md). The substrate was o-phenylenediamine (0.4 mg/mL) and hydrogen peroxide (0.015%) in citrate phosphate buffer.

**Definition of reticulocyte count nadir.** To compare the time course of illness among different patients, we selected the day of the lowest documented reticulocyte count, ie, the reticulocyte count nadir, as the reference point. This day was called “day 0,” and subsequent days were called +1, +2, +3, etc, while the days preceding nadir were called −1, −2, −3, etc, respectively.

**RESULTS**

**Subject description.** During the 5½-month period beginning March 13 and ending August 23, 26 patients with underlying hereditary hemolytic anemia developed acute, profound red cell aplasia. The peak incidence occurred in April 1984. In contrast, during the preceding 3 years there had been no reported cases of red cell aplasia in patients with hemolytic anemia. Fourteen patients were males and 12 were females. The patients were 2 to 23 years old (median 11 years), with

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**Fig 1.** The nadir values of hematocrit and reticulocyte count in 26 patients with acute red cell aplasia. Solid circles: patients with sickle cell disease. Open circles: patients with other types of hemolytic anemia. The oval areas represent the baseline values of these patients.
The patients with the three milder cases of anemia with nadir hematocrits of 26%, 28%, and 32% all had SC (Fig 1).

Bone marrow examinations performed in 3 patients 0 to 2 days after the reticulocyte count nadir revealed moderate to severe erythroid hypoplasia with few immature erythroid precursors. Cellularity and numbers of precursors in all other cell lines were normal. Erythropoiesis recovered rapidly in these patients with a reticulocytosis of 5% or more within 3 to 5 days.

The natural course of red cell aplasia is illustrated in one patient who refused blood transfusion on religious grounds (Fig 2). The life-threatening anemia with severe hypoxic symptoms was corrected by a sudden, spontaneous bone marrow recovery and a burst of nucleated red cells in the circulation, with reticulocyte count peaking at 44% (Fig 2).

Reticulocytosis occurred within 2 to 14 days of presentation and 2 to 8 days of the reticulocyte count nadir in all of the patients. In 5 patients we had the opportunity to document the true reticulocyte count nadir during the hospitalization by demonstrating an initial fall in the reticulocyte count. In all 5 patients, the duration of the reticulocytopenia from the true nadir to a reticulocyte count level of at least 2.5% was 6 to 8 days.

Platelets. No arrest of platelet production was demonstrated. The platelet count dropped below 150 × 10^9/L in only two patients. Marrow recovery was associated with thrombocytosis. Platelet count peaks over 500 × 10^9/L (range 509 to 1133 × 10^9/L) were seen in 13 patients, a median of day +4 from the reticulocyte count nadir.

White Blood Cells. Leukopenia usually did not occur. In contrast, many patients developed leukocytosis, in 9 patients exceeding 25 × 10^9/L and ranging up to 47.3 × 10^9/L (corrected for nucleated red cells) in association with early marrow recovery. A subgroup of patients presented with atypical lymphocytosis (over 10% in differential in 5/23), eosinophilia (over 5% in 9/23), a short-lived neutropenia (absolute neutrophil count below 1000 × 10^6/L in 4/23), and appearance of circulating plasma cells up to 5% (4/23). In three patients no differential white cell count was available. These changes in white blood cells tended to occur in the same individuals: 26% (6/23) had 2 to 4 of the above-mentioned characteristics. The changes were short-lasting and were observed very close to the reticulocyte count nadir (days −1 to +1).

Therapy in acute red cell aplasia. As treatment for the acute red cell aplasia and severe anemia, 22 of 26 patients received red cell transfusions; six were total or partial exchange transfusions. One patient refused blood transfusion on religious grounds (Fig 2), and 3 patients with hemoglobin SC disease did not require transfusion (Fig 1). In addition, the patients received intravenous fluids and symptomatic pain medication. Some were initially started on antibiotics because of suspected bacterial infection; however, no infections could be documented. The hospitalizations were in general short (median 4 days, range 2 to 13 days) and uneventful.

Virologic studies. Acute-phase sera were available for study in 21 patients. (In the remaining five cases the first serum sample was obtained at day +35 or later after the reticulocyte count nadir.) In five acute-phase sera, 10^9 to 10^13 viral particles/mL were detected by electron microscopy (Fig 3). The purified virions of one acute-phase serum sample were found to contain 5.4 kb linear DNA species in 110 S particles physically indistinguishable from prototype B19 or other parvoviruses.11,12

B19-DNA, which was demonstrated by DNA hybridization, was detected in high concentration (>1000 ng/mL) in 7 acute-phase sera of 5 patients, and in low concentration (0.3 to 10 ng/mL) in the sera of an additional 8 patients (Fig 4). Serum samples with high-concentration B19-DNA and viral particles demonstrated by electron microscopy were drawn between day −5 to day +1 from the reticulocyte count nadir. Low-concentration antigenemia was detectable up to day +4 (Fig 4). In patients who were viremic, virus was also demonstrated in urine and throat gargle.16

Serologic studies. B19-specific IgM was detected in the sera of 24/26 patients. The highest titers were observed at

![Fig 2. Natural course of aplastic crisis with sudden, spontaneous recovery in an 18-year-old patient who refused transfusion. Day 0 indicates reticulocyte count nadir. BM = bone marrow examination, in which the erythroid cell line was represented by only a few proerythroblasts.](image)

![Fig 3. Aggregate of parvovirus-like particles in acute-phase serum from a patient with aplastic crisis. The particles are negatively stained with uranyl acetate. Original magnification ×156230; current magnification ×107,798.](image)
The reticulocyte count nadir and during the subsequent 10 days. The titers stayed at detectable levels for about 140 days (Fig 5). In six patients with viral antigenemia at the nadir, B19-specific IgM seroconversion was demonstrated.

B19-specific IgG was negative at the reticulocyte count nadir in all patients (Fig 5), but thereafter rose to high concentrations and tended to stay high for 4 to 5 months (Fig 5). The earliest positive specific IgG was detected on day +1 after the reticulocyte count nadir, and on day +2 after the true nadir. Follow-up was insufficient to determine the duration of IgG antibody positivity. B19-specific IgG was detected in 21 of the 22 patients with convalescent-phase sera available. Fourteen patients demonstrated a fourfold or greater rise in IgG antibody.

In 24 out of our 26 cases we had evidence of human parvovirus B19 infection; 24 had specific IgM detected, and 13 of these also had B19-DNA demonstrated in acute-phase serum. In two cases, no evidence for B19 infection could be obtained. Both of these patients, however, fit our criteria for aplastic crisis. For one patient, studied on day +1, convalescent-phase serum was not available. Sera from the other patient, obtained on days +1 and +29, were negative for virus and for specific IgM and IgG antibodies.

Inhibition of erythroid colony formation. The 5 acute-phase sera that demonstrated high-concentration B19-DNA and parvovirus particles on electron microscopy inhibited erythroid colony formation in vitro. The colony-forming units-erythroid (CFU-E)-derived colonies were completely inhibited at serum dilutions of 1:10 and 1:100 (Fig 6). The more primitive burst-forming units-erythroid (BFU-E)-derived colonies were inhibited completely at 1:10 and partially at 1:100 serum dilution (Fig 6). The sera retained strong inhibitory effects on CFU-E and BFU-E after being heated to 56 °C. Parvovirus-negative sera, screened blindly, were not inhibitory in the erythropoiesis assay. The parvovirus-negative acute sera were comparable to the positive ones regarding the previous blood transfusion status of the patients. The colony-forming units, granulocyte-macrophage (CFU-C)-derived colony growth was not affected by viremic sera.

DISCUSSION

This is the first large epidemic of pure red cell aplasia reported in the United States. In the preceding 3 years, no children with aplastic crisis were diagnosed in northeastern Ohio. Our series consisted of 24 children and 2 young adults. Two previous reports from the United States included 6 adult patients during 1980 to 1982 in Illinois, and 6 children during 1979 to 1983 in Washington, D.C. Occurrence of aplastic crisis is thought to confer lasting immunity, because the illness usually affects children under 16 years of age, and more than one episode during a lifetime is rare. Aplastic crises have been previously documented in a variety of hemolytic anemias, eg, sickle cell disease, hereditary spherocytosis, hereditary elliptocytosis, thalassemia major, thalassemia intermedia, hemoglobin E/thalassemia, pyruvate kinase deficiency, paroxysmal noc-
disease, sickle-beta-thalassemia, and hereditary spherocytosis. The underlying hereditary hemolytic anemias that occur during aplastic crisis, but little attention has been paid to the white blood cells or the platelets. It is generally thought that these cells are not affected. Slight thrombocytopenia and anemia at hospital admission. The duration of profound reticulocytopenia was 6 to 8 days in the 5 cases in whom the true nadir of the reticulocyte count could be documented during the hospitalization. The literature offers detailed enough case reports of a few cases only,13,18,25 but in those the total duration of the reticulocytopenia also was very consistently 6 to 7 days. The whole course of the illness, from the onset of prodromal symptoms to the reappearance of circulating reticulocytes, is about 10 to 12 days.4

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of considerable interest that fetal infection with the B19 parvovirus has recently been associated with human hydrops fetalis and late intrauterine fetal demise. B19 virus has now also been reported in association with acute polyarthritis and prolonged arthropathy in adults. In addition, speculation has recently been raised about a possible parvoviral etiology of rheumatoid arthritis.

The present knowledge suggests that acute red cell aplasia in patients with underlying hereditary hemolytic anemia is probably the most serious and life-threatening illness caused by human parvovirus B19. Work must continue to elucidate the complete spectrum of human disease caused by this agent.

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