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 profound 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, 10^6 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.

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A PLASTIC CRISIS in a patient with underlying hereditary hemolytic anemia is defined as a transient episode of pure red cell aplasia, with virtual absence of erythroid precursors in the bone marrow and with the absence of reticulocytes in the circulation. Because of the shortened red cell life span in hereditary hemolytic anemia, a temporary interruption of erythropoiesis leads to a precipitous fall in hematocrit and acute, life-threatening anemia. Recovery is heralded by a spontaneous, massive increase of erythropoiesis, also indicated by a burst of reticulocytes in the peripheral blood.1,3

The infectious nature of aplastic crisis in patients with hemolytic anemia has been suggested by reports both of family outbreaks and of clustering of attacks in time.4,5 Epidemics have been reported from Jamaica, where outbreaks of aplastic crisis have been seen cyclically at about 5-year intervals.6 The last Jamaican epidemic occurred during 1979–1980 with 38 identified cases.6 A cluster of cases occurred simultaneously in England.7,8 Although an infectious etiology was suspected when aplastic crisis in hereditary hemolytic anemia was first described,1 it took over 30 years to obtain information about the causative agent. Recent studies have associated aplastic crisis with human parvovirus infection.9,10

During 1984 in northeastern Ohio we investigated a large epidemic of red cell aplasia involving 26 patients with hereditary hemolytic anemia. In this report we characterize the course of the illness and its pathogenesis, and present evidence indicating the etiologic role of human parvovirus B19 infection in the epidemic.

MATERIALS AND METHODS

Patients. In March 1984, three patients with sickle cell disease presented to Rainbow Babies and Childrens Hospital in Cleveland with profound anemia and reticulocytopenia. These cases prompted the development of a detailed, prospective study to investigate the etiology of aplastic crisis.

To identify as many cases of aplastic crisis as possible, we contacted all pediatric hematologists and most internist hematologists in northeastern Ohio, the American Sickle Cell Association in Cleveland, and all major health care providers for patients with sickle cell disease in Cleveland, as well as physicians with large black practices in Cuyahoga County.

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 at 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.10,11 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 DNA12 inserted in the SP65 riboprobe vector. Probe synthesis and hybridization were performed as described by Zinn et al10 and B19 DNA concentrations estimated by densitometric comparison with standards constructed by dilution, in negative serum, of purified 110S B19 virions,13 with extinction coefficients derived for the minute virus of mice, another parvovirus.12 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.11 The virions from one case were purified and compared with other parvoviruses.12

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.13,14 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 radioimmunoassay method.15 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-B1) 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, i.e., 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. (Fig 1). The nadir reticulocyte counts, which ranged from 0% to 2.2%, were below 1% in 85% (22/26) of the cases. The average baseline reticulocyte counts were 8.3% to 24.4% in SS and 3.2% to 5.8% in SC and S-thal patients (Fig 1). Severe anemia (Hct of below 20%) was documented in 23 of the 26 patients. The nadir hemoglobin values were 7% to 18% in 55 (average baseline 19% to 28%) and 17% to 32% in SC and S-thal (average baseline 27% to 30%).

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**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

![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.](image-url)
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 x 10^9/L in only two patients. Marrow recovery was associated with thrombocytopenia. Platelet count peaks over 500 x 10^9/L (range 509 to 1133 x 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 x 10^9/L and ranging up to 47.3 x 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 x 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.

Serologic studies. B19-specific IgM was detected in the sera of 24/26 patients. The highest titers were observed at
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, convales-
cent-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 parvovi-
rus-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-
Epidemic red cell aplasia

Interest has been focused on the red blood cell changes 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 slight leukopenia have occasionally been observed. Although we did not see thrombocytopenia or leukopenia, a subgroup of patients presented with atypical lymphocytosis, eosinophilia, neutropenia, and circulating plasma cells. These characteristics tended to occur in the same individuals, and they also tended to occur very close to the reticulocyte count nadir. There are occasional reports of atypical lymphocytes, eosinophilia, plasma cells, or neutropenia occurring during aplastic crisis, but usually not in combination. The white blood cell changes may be very short in duration, and because the true reticulocyte count nadir has usually passed when the patient is admitted to hospital, the characteristic white cell changes may have been missed. On the other hand, at the marrow recovery stage, the burst of erythropoiesis was preceded by leukocytosis and thrombocytosis in many of our patients.

Evidence of human parvovirus B19 as the etiologic agent was obtained by demonstrating parvovirus-like particles by electron microscopy in five acute-phase sera, and by demonstrating B19-specific DNA by hybridization in high concentration in the sera of eight additional patients. The high-concentration viremia was observed during a very short period before and at the reticulocyte count nadir, ie, from day -5 to day +1 (Fig 4). The time of onset and the duration of viremia before the manifestation of aplastic crisis is not known. One of our cases with high-concentration viremia on days -5 and -4 had nonviremic serum samples available from days -16 and -13 (Fig 4). We cautiously conclude that viremia is unlikely to occur before the appearance of prodromal symptoms.

As further evidence of parvovirus B19 infection, B19-specific IgM was detected in sera of 24/26 patients, of whom six seroconverted. B19-specific IgM was detectable for about 140 days (Fig 5), compared to the 10-week estimate previously given. Specific IgG was detectable in convalescent-phase sera of 21/22 patients, including 14 seroconversions. Specific IgG stayed detectable throughout the follow-up (Fig 5); IgG is known to be measurable for 6 months or longer. Based on these data, 24 of the 26 patients with epidemic red cell aplasia had evidence of B19 infection.

The five acute-phase viremia sera with viral particles seen by electron microscopy and B19-DNA in high concentration all inhibited erythroid colony growth in vitro. CFU-E-derived colonies appeared more sensitive to the inhibitory effect of viremic sera than the BFU-E-derived colonies. CFU-C-Derived colonies were unaffected. These data are consistent with direct effect of the virus on erythroid progenitor cells in bone marrow as the pathogenetic mechanism in epidemic red cell aplasia. Partially purified parvovirus has been shown to inhibit proliferation of isolated erythroid progenitor cells of the CFU-E stage of development, and parvovirus has been demonstrated in erythroid progenitor cells infected in vitro by specific immunofluorescence and electron microscopy.

Human parvovirus B19 seems to be widespread throughout the world. B19-specific IgG has been detected in 25% of normal blood donors in France, in over 40% in Australia, and in 61% in England. The effects of B19 do not appear to be restricted to bone marrow erythroid aplasia. Recent studies suggest that a mild acute exanthematosus disease in children, called “fifth disease” or erythema infectiosum, is also caused by human parvovirus. Fifth disease outbreaks have been recently reported from England, Japan, Finland, and Manitoba, Canada. Most interestingly, during our epidemic of red cell aplasia in 1984 in northeastern Ohio, an epidemic of fifth disease with over 450 reported cases occurred concurrently in the same geographic area.

In vitro studies have shown that the autonomously replicating parvoviruses require their host cell to traverse the S-phase of the mitotic cycle for viral replication to proceed. Thus parvoviruses would be expected to grow to high concentrations in rapidly replicating cells such as those of hyperplastic bone marrow or the tissues of the developing fetus. Indeed many parvoviruses have been shown to be teratogenic and fetocidal in their animal hosts. For example, the porcine parvovirus is well recognized as a cause of reproductive failure due to intrauterine fetal death.
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 polyarthitis 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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