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, $10^9$ 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.

**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.

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. Epidemics have been reported from Jamaica, where outbreaks of aplastic crisis have been seen cyclically at about 5-year intervals. The last Jamaican epidemic occurred during 1979-1980 with 38 identified cases. A cluster of cases occurred simultaneously in England. Although an infectious etiology was suspected when aplastic crisis in hereditary hemolytic anemia was first described, it took over 30 years to obtain information about the causative agent. Recent studies have associated aplastic crisis with human parvovirus infection.

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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patients were 2 to 23 years old (median 11 years), with hemolytic anemia.

April 1984. In contrast, during the preceding 3 years there was a profound red cell aplasia. The peak incidence occurred in the 3 weeks ending March 13 and beginning August 23, 1984, with 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.

RESULTS

Subject description. During the 5½-month period beginning March 13 and ending August 23, 1984, 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 incidence peaks of 3 and 14 years. The subjects included two sibling pairs and one mother-and-child pair. The time difference in the onset of illness as defined by the reticulocyte count nadir was 8 and 12 days in the two sibling pairs. The mother and child contracted the illness simultaneously.

The underlying hereditary hemolytic anemia was sickle cell disease (SS) in 20, hemoglobin SC disease (SC) in 4, sickle-beta-thalassemia (S-thal) in 1, and hereditary spherocytosis (HS) in 1 patient. The patient with HS was white; the other 25 were black.

Symptoms of illness. All patients had prodromal symptoms of illness for 1 to 17 days (median 4 days) prior to the manifestation of red cell aplasia. All patients reported fever, chills, lethargy, or malaise. Abdominal symptoms were reported by 85%; 58% had nausea or vomiting, 54% had abdominal pain, and 12% had diarrhea. Seventy-three percent suffered from a variety of aches and pains: 46% had headache, 27% had back pain, 27% had arthralgias, and 15% had myalgias or diffuse achiness. In addition, 42% had mild respiratory symptoms, consisting of rhinorrhea (23%), cough (19%), or conjunctivitis and photophobia (15%). Twenty-three percent had a skin rash. A skin eruption was observed in two patients during hospitalization. When questioned directly for dermatologic symptoms, four other patients reported having had a skin rash prior to hospital admission. The rashes had involved arms, face, legs, or trunk, and had been faint and maculopapular. The duration of rash was 3 to 21 days. All symptoms of illness resolved during hospitalization, usually within 3 to 5 days.

Red cell aplasia. The prominent hematologic feature was red cell aplasia, manifested as acute, severe anemia and profound reticulocytopenia (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 hematocrits were 7% to 18% in SS (average baseline 19% to 28%) and 17% to 32% in SC and S-thal (average baseline 27% to
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 $150 \times 10^9$/L in only two patients. Marrow recovery was associated with thrombocytosis. Platelet count peaks over $500 \times 10^9$/L (range $509$ to $1133 \times 10^9$/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 $25 \times 10^9$/L and ranging up to $47.3 \times 10^9$/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 $1000 \times 10^6$/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-url)

![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 $\times 156230$; current magnification $\times 107,798$.](image-url)
I:

0.31

PARVOVIREMIA

-15 -10 -5 0 5 10 15 20 25 30 3 40

DAYS FROM RETICULAR NADIR

-20 0 20 40 60 100 130 200

1:21

Fig 5. B19-specific IgM and IgG antibody titers related to the time from the reticulocyte count nadir (− day 0). Individual values of 26 patients (1 to 5 values per patient) are plotted, and the average courses are indicated by the curves (modified after linear regression lines over three times intervals per curve).

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.

Fig 6. Inhibition of erythroid colony growth by human parvovirus B19. CFU-E's and BFU-E's were assayed in the presence of viremic sera, and measured as percentage of colony growth of the control. Serum dilution curves of four patients (indicated by different symbols) with the highest viremia peaks (over 40,000 ng of virus per ml serum) are shown.
ternal hemoglobinuria, and acquired hemolytic anemias. The underlying hereditary hemolytic anemias observed in our series were sickle cell disease, hemoglobin SC disease, sickle-beta-thalassemia, and hereditary spherocytosis. Although aplastic crisis is not associated with a certain type of hereditary hemolytic anemia, the severity of the aplastic crisis correlated with the severity of underlying hemolytic anemia; 3 of our 4 cases with hemoglobin SC disease did not require red cell transfusions (Fig 1).

The incubation period of epidemic red cell aplasia suggested in the literature has been 9 ± 3 days or 17 days. The incubation times observed in the two sibling pairs in our study were 8 and 12 days, in agreement with the shorter estimate. The prodromal symptoms of illness included fever, pains and aches, abdominal symptoms, and mild respiratory symptoms. Associated skin rash was reported in 23%. A very similar pattern of prodromal symptoms has been described repeatedly since the first reports of aplastic crisis in hemolytic anemia. A skin rash was rarely described in these reports. Because most of the patients were black, a rash might not have been easily detected; however, in Mortimer’s review of spherocytosis in white patients, only one out of 64 had a skin rash.

The prodromal symptoms were followed by a precipitous fall of hematocrit and detection of severe reticulocytopenia 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, but in those the total duration of the reticulocytes 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.

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 in general, 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 true 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 5 acute-phase sera, and by demonstrating B19-specific DNA by hybridization in high concentration in the sera of 8 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 6 seroconverted. B19-specific IgM was detectable for about 140 days (Fig 5), compared to the 10-week estimate previously given. B19-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 exanthematous 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 fetotoxic 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 polyarthritid 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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Human parvovirus B19-induced epidemic acute red cell aplasia in patients with hereditary hemolytic anemia

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