The Role of Parvovirus B19 in Aplastic Crisis and Erythema Infectiosum (Fifth Disease)

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In 1984, simultaneous outbreaks of aplastic crisis and erythema infectiosum occurred in northeastern Ohio. Sera were analyzed from 26 patients with aplastic crisis: 24 had IgM specific for parvovirus B19, five had B19-like particles by electron microscopy, and 13 had DNA from B19; no sera from 33 controls had evidence of recent infection with B19 (P < .0001). DNA from B19 was also detected in specimens of throat gargle and urine from two patients with aplastic crisis. Sera from 36 of 51 children with erythema infectiosum had B19-specific IgM, compared with serum from one of 42 susceptible controls (P < .0001). DNA from B19 was detected in sera from only two of 51 patients who had erythema infectiosum. The secondary attack rates among susceptible contacts decreased with age (overall total, 49.6%). Differential rates of asymptomatic infection were observed among black (68.8%) and white (20.0%) household members (P = .003). These were the first identified simultaneous outbreaks of aplastic crisis and erythema infectiosum. Their occurrence provided an opportunity to study the epidemiology and spectrum of B19 infection with geographically and temporally matched comparison groups; our results support the hypothesis that infection with parvovirus causes these two clinical entities.

An infectious cause for acquired pure red cell aplasia (aplastic crisis) in patients with chronic hemolytic anemias has long been suspected because of community epidemics [1] and family clusters [2-6] of aplastic crises, and because aplastic episodes usually occur only once in a lifetime [1]. Erythema infectiosum (EI) has also been thought to have a single infectious cause [7], but numerous attempts to isolate an agent from patients with naturally occurring EI have failed. In 1981, infection with human parvovirus B19 was first associated with aplastic crisis [1], and in 1983, infection with B19 or similar parvovirus was associated with EI [8]. Numerous other case reports and studies have also associated recent B19 infection with aplastic crisis [1, 9-19] or EI [8, 18-23].

E. Palmer for electron microscopy; Dr. S. Cotmore for hybridization studies; C. Tsou and Drs. H. Wulff and J. Stewart for serological analyses; J. Moore for erythropoiesis inhibition assays; C. Ivey, R. Odum, and D. Kotlovker for data preparation and programming assistance; D. Donovan, M. Parvin, C. Beck, D. Arinson, J. Davis, and S. Price for specimen collection and management; A. Navin, L. Grabbe, D. Collier, and Drs. A. Friede and R. Gunn for critical review of this manuscript.

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In 1984, we investigated concurrent epidemics of aplastic crisis and EI in Ohio. These simultaneous outbreaks allowed us to determine the strength of the association between each of these clinical entities and B19 infection by using matched comparison groups and to study the epidemiology and clinical spectrum of B19 infection in a defined population.

Subjects and Methods

Investigation of aplastic crisis. Background. The investigation was initiated when aplastic crises were diagnosed in eight patients with chronic hemolytic anemias who were admitted to northeastern Ohio hospitals within a six-week period. Three contiguous counties were involved in the investigation: Cuyahoga (which includes the entire city of Cleveland), Summit, and Lorain. There are no reliable data regarding the incidence of aplastic crisis in previous years or the prevalence of hemolytic anemias in the Cleveland area. The most common chronic hemolytic anemia in this area is sickle cell disease, i.e., either homozygous hemoglobin S (SS) disease or double heterozygous hemoglobinopathy—SC disease or S-β-thalassemia. We used estimates of the prevalence of sickle cell disease in Cincinnati to estimate the prevalence of aplastic crisis in previous years or the prevalence of hemolytic anemias in the Cleveland area. The study area, given the assumption that the black populations of the two cities [24] have similar prevalences of sickle cell disease.

Definition and identification. We defined a case of aplastic crisis as a decreased reticulocyte count (<50% of the baseline value), lasting less than two weeks, in a patient with a chronic hemolytic anemia. The baseline value was defined as the average reticulocyte count observed in up to three visits to the clinic (while healthy) within the previous two years; if this value was not available, we used the reticulocyte count obtained after the aplastic crisis (at least two months after discharge from the hospital).

To identify cases of aplastic crisis, we contacted local physicians, neighborhood clinics, and hospital outpatient departments throughout northeastern Ohio and all major hospitals and clinics caring for patients with sickle cell disease in Ohio; we also reviewed registries of patients admitted to hospitals in northeastern Ohio since January 1981.

Study design. The 26 patients from northeastern Ohio who had an aplastic crisis during 1984 were compared with controls randomly selected from a pediatric population of ~300 nonsplenectomized patients who had a chronic hemolytic anemia with no history of aplastic crisis (according to clinical records and information from parents) and who had been monitored during the previous two years in clinics associated with the hospitals to which the patients with aplastic crisis had been admitted. Of the 69 potential controls, three subsequently developed aplastic crisis and were recategorized as patients, 17 could not be contacted because of telephone or address change, and two had a parent or guardian who refused to allow participation (response rate, 50 [75%] of 66). Of the remaining 47 controls, 44 were black, two were white, and one was Asian. Twenty-six of the controls had SS disease; nine, SC disease; four, S-β-thalassemia; one, hereditary spherocytosis; one, homozygous hemoglobin H disease; one, homozygous hemoglobin C disease; one, pyruvate kinase deficiency; one, glucose-6-phosphate dehydrogenase deficiency; and one, β-thalassemia major. The parents or guardians participated in structured interviews regarding demographics, socioeconomic status, household population density.
Parvovirus B19 Infection

Definition and identification. EI was first established as a clinical entity on the basis of outbreaks in the late winter and early spring of 1924 and 1925 in Cuyahoga County, Ohio [25]. Outbreaks of EI in schools were reported to the measles surveillance system of the Cuyahoga County Board of Health in 1969 (65 cases), 1970 (109 cases), 1973 (1,180 cases), 1981 (113 cases), and 1983 (32 cases); in each year, most of the cases occurred in late winter and early spring. A total of 85 cases (median, 6 cases; range, 0–13 cases) were reported in the other 14 years between 1965 and 1982. By the time the outbreak of aplastic crisis was first noted, cases of EI in schools had been reported through the measles surveillance system; surveillance efforts were increased by the County Board of Health in late January 1984 to document the extent of the EI outbreak.

Definition and identification. To describe the timing and extent of the EI outbreak, we used all cases of EI reported to the measles surveillance system by county schools. For the serological study, we considered only pediatric patients having rash consistent with EI [7, 25] that was diagnosed between May 1984 and July 1984 by physicians affiliated with the hospitals to which the patients with aplastic crisis had been admitted.

Study design. Blood samples were obtained from randomly chosen patients with EI and from pediatric controls without rash seen in the same clinics between May 1984 and July 1984. The parents or guardians of 36 randomly selected patients from the serological study were interviewed to obtain data on the recent medical history of household contacts, and a blood sample was obtained from all household contacts. We defined a household contact as anyone (other than the index patient with EI) residing in the same house during the 90 days before the onset of rash in the patient.

Statistical analysis. The serological results were categorized as positive or negative. For dichotomous factors, we used the method of Thomas and Gart [26, 27] to obtain the odds ratio and the 95% confidence interval; Fisher’s two-tailed exact test was used to determine probability [28]. Nondichotomous (continuous) factors were analyzed by the Wilcoxon rank-sum test [29]. The significance level for all statistical analyses was \( P \leq .05 \).

We used linear logistic regression to examine the association between aplastic crisis and possible risk factors [30]; because of the small amount of data, it was necessary to limit regression models to two or three risk factors. Each individual hematocrit was categorized as above or below the median hematocrit (26.5) of the combined group of patients and controls. Serological data from the outbreaks of aplastic crisis and EI were combined, and persons <20 years of age were divided into age groups covering five-year periods; the test for linear trend in proportions [31] was used to describe the relation of seropositivity (IgM and IgG) to age.

Laboratory analysis. Specimens. Acute- and/or convalescent-phase serum samples were collected from all 26 patients with aplastic crisis, and sera were collected from 33 (70%) of the 47 controls. Samples of urine and/or PBS throat gargle specimens were obtained from six patients within a day of diagnosis. Sera were obtained from 71 (89%) of 80 household contacts of the 26 patients with aplastic crisis, including at least one household contact of each patient with aplastic crisis. Sera were also obtained from 49 (67%) of 73 household contacts from 31 (62%) of the 50 households of the IgG-seronegative controls with sickle cell disease and from 32 (89%) of 36 household contacts from 10 (77%) of the 13 households (total, 53 household contacts) of the controls who did not have sickle cell disease or who were IgG-seropositive.

We obtained sera from 51 pediatric patients with EI and 56 pediatric controls. All the patients were
white; of the controls, 51 were white, three were black, and two were Hispanic. Blood samples were drawn from five of the 51 patients during the two- to nine-day period before the rash developed. Sera were also obtained from household contacts (93 [83%] of 108) of 32 of the 51 patients with EI.

All serum, urine, and PBS gargle specimens were stored at −70 C.

**Diagnostic techniques.** The sera were assayed in a blinded fashion for B19 DNA by modification of a dot hybridization procedure [32] and for B19-specific IgM and IgG with a capture ELISA, both described elsewhere [33]. Urine and PBS gargle were also evaluated by the hybridization procedure. In this procedure, 0.5-μl aliquots of serum or 45-μl aliquots of urine or PBS gargle were digested with 100 μg of proteinase K/ml for 30 min at 60 C; an equal volume of 0.6 N NaOH was then added, and the sample was hydrolyzed at 60 C for another 30 min. An equal volume of 2 M ammonium acetate (pH 7.0) was added, and the sample was applied to premoistened nitrocellulose filters with a slot-blower apparatus (Schleicher and Schuell, Keene, NH). The slot blots were probed with 32P-labeled RNA having a high specific activity that had been synthesized in vitro from a full-length copy of B19 DNA [34] inserted in the SP65 riboprobe vector. Probe synthesis and hybridization were performed as described by Zinn et al. [35]. Concentrations of B19 DNA were estimated by densitometric comparison with standards, which consisted of dilutions (in negative serum) of purified 110S B19 virions [34]; the extinction coefficients used were those derived for another parvovirus, the minute virus of mice [36]. The limits of detection were 0.30 ng/ml for serum and 0.003 ng/ml for urine and PBS gargle. The viral DNA from acute-phase sera of patients with aplastic crisis was compared with prototype DNA from B19 on alkaline agarose gels [34]; the virions from one patient were purified and compared with other parvoviruses by methods described previously [36].

Acute-phase sera from patients with aplastic crisis were also evaluated in a blinded fashion for B19-like particles by negative-stain electron microscopy with uranyl acetate stain and for inhibition of erythroid colony formation in vitro [37]. Nine paired (acute- and convalescent-phase) sera from patients with aplastic crisis were also evaluated for the presence of antibodies in a blinded fashion by the following methods: (1) CF for antibody to parainfluenza virus types 1, 2, and 3, adenovirus, respiratory syncytial virus, and *Mycoplasma pneumoniae* [38]; (2) IHA for antibody to cytomegalovirus [39] and herpes simplex virus types 1 and 2 [40]; and (3) indirect immunofluorescence for antibody to Epstein-Barr virus [41] and varicella zoster virus [42].

Because some cases of EI may be clinically indistinguishable from early rubella, rubella serology by HAI [43] was obtained for the 13 patients with EI from whom paired sera were available.

**Results**

*Outbreak of aplastic crises. Epidemiological studies.* From 13 March 1984 to 23 August 1984, 26 patients with aplastic crisis were admitted to hospitals participating in this study (figure 1): 23 were admitted in Cleveland, two in Akron, and one in Lorain. Twenty patients had SS disease; four, SC disease; one, S-β-thalassemia; and one, hereditary spherocytosis. None had a history of aplastic crisis or splenectomy. No other cases of aplastic crisis were identified between 1980 and mid-1984 in northeastern Ohio. The estimated risk of aplastic crisis among patients with sickle cell disease in Cuyahoga, Summit, and Lorain counties in 1984 was 2.4% (25 cases of aplastic crisis among 1,107 cases of chronic hemolytic anemia). The estimated risks for patients with SS disease, SC disease, or S-β-thalassemia are presented in table 1.

Fourteen of the patients were male, and 12 were female. The median age was 11.4 years (range, 2.3–23.5 years). The 25 patients with sickle cell disease were black, and the patient with hereditary spherocytosis was white. The residences of the 23 patients from Cleveland who had sickle cell disease had a distribution similar to that of a random list of addresses of patients from Cleveland who had sickle cell disease. The 26 patients were from 23 households; only six patients (two pairs of siblings and a mother-child pair) had known contact with another patient with aplastic crisis (i.e., the respective relative). The clinical features of the patients are presented elsewhere [33]. Although a rash was present on six patients just before or during aplastic crisis, no patients had the rash that is diagnostic of EI. None of the patients with aplastic crisis or the controls had a history of EI.

When we restricted patients and controls to those with sickle cell disease, the controls had significantly higher baseline hematocrits (table 2). This relation did not change if we further restricted the analysis.
Figure 1. Epidemic curves for patients with aplastic crisis (upper graph, \( n = 26 \)) and patients with erythema infectiosum (lower graph, \( n = 452 \)); Cuyahoga County, Ohio, December 1983–August 1984.

to patients and controls with SS disease (16 patients: median hematocrit, 23.4 [range, 20.0–28.5]; 17 controls: median hematocrit, 26.0 [range, 23.0–33.6]; \( P = .02 \)). The presence of a B19-specific, IgM-seropositive household contact was significantly associated with aplastic crisis (9 of 17 patients, 0 of 14 controls; \( P = .001 \)). The other risk factors associated with aplastic crisis were sharing eating utensils with other household members (odds ratio, 5.1; confidence interval, 1.20–22.2; \( P = .014 \)) and recent contact with a dog (odds ratio, 5.1; confidence interval, 1.27–21.1; \( P = .014 \)) or a cat (odds ratio, 5.8; confidence interval, 1.19–31.2; \( P = .015 \)). Household population density, household contacts of elementary school age (5–13 years old), the presence of a recently ill household contact who had three or more common symptoms of a viral illness, recent contact with an unimmunized dog or cat, or a recent rash were not significantly associated with the occurrence of aplastic crisis. Recent use of antibiotics was significantly more common among controls (17 of 34) than among patients (4 of 22, \( P = .024 \)), although no significant associations were found with recent transfusion or use of folate, aspirin, acetaminophen, codeine, or meperidine. Multivariable analyses revealed that only sharing eating utensils and recent contact with a dog or cat were independently associated with aplastic crisis. Although the controls were younger than the patients, these associations were not affected when age was taken into account by using logistic regression. The presence of an IgM-seropositive household contact was not included as a factor in the initial multivariable analyses because it was not known whether the patient with aplastic crisis or another member of the household was the index case of B19 infection in individual households.

Table 2. Demographic and clinical features of patients with aplastic crisis and control subjects with sickle cell disease, Ohio, 1984.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Patients with aplastic crisis</th>
<th>Controls</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>11</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>11</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>22, 9.6 (2.6–18.1)</td>
<td>34, 4.7 (0.8–22.7)</td>
<td></td>
</tr>
<tr>
<td>Baseline hematocrit</td>
<td>21, 24.3 (20.0–34.4)</td>
<td>27, 27.0 (23.0–35.1)</td>
<td>0.022</td>
</tr>
<tr>
<td>Baseline reticulocyte count</td>
<td>20, 9.7 (2.8–22.5)</td>
<td>24, 12.1 (2.1–22.7)</td>
<td></td>
</tr>
<tr>
<td>Baseline level of hemoglobin</td>
<td>11, 8.4 (7.8–11.4)</td>
<td>26, 9.6 (8.0–12.4)</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. NS = not significant. Probability was determined by using the Wilcoxon rank sum test [29]. Data are given only for index patients and controls.
or whether mutual IgM seropositivity within households reflected a common exposure; however, when this factor was included in multivariable analyses, only the presence of an IgM-seropositive household contact and recent contact with a cat were independently associated with having an aplastic crisis.

**Laboratory analysis.** In acute-phase sera from 24 patients with aplastic crisis, DNA hybridizing with the 5.4-kilobase DNA fragment of prototype B19 was detected in concentrations of 4,000–68,000 ng/ml in five (21%) of the patients and 0.3–4.2 ng/ml in eight (33%) of the patients. The hybridizing DNA was contained within a 110-S particle physically indistinguishable from prototype B19 and other parvoviruses. DNA from B19 was not detectable in the other 11 acute-phase sera from patients, nor in 31 of 33 sera from controls (P < .0001). DNA from B19 was also detected in concentrations of up to 1.1 ng/ml in the urine and 11 ng/ml in PBS gargle from two patients whose sera had concentrations of B19 DNA >25,000 ng/ml, but DNA from B19 was not detected in the urine and PBS gargle from four other acute-phase sera from patients with aplastic crisis, one of whom had serum with a B19 DNA concentration of 4,000 ng/ml. The five sera with a high concentration of B19 DNA markedly inhibited erythroid colony formation in vitro, a property that was retained after the sera were heated to 56 °C for 30 min; only these five sera had B19-like particles that were revealed by electron microscopy. No other virus particles were revealed by electron microscopy [33].

Of the 26 patients with aplastic crisis, 24 (92%) had an acute- or convalescent-phase serum specimen that had B19-specific IgM, compared with none of 33 controls (odds ratio, ∞; P < .0001). This relation did not alter when we excluded participants who did not have sickle cell disease, patients who were not the index case in their households, and controls who had evidence of prior infection with B19 (20 of 22 patients, none of 22 controls). B19-specific IgG was found in sera from three of the four people excluded as controls because they had a previous aplastic crisis and in sera from only five of 33 controls with no history of an aplastic crisis (P = .026).

When serum specimens from 56 household contacts (who did not have an aplastic crisis) of patients with aplastic crisis were examined, only one contained DNA from B19, at a concentration of 2 ng/ml. This IgM-positive specimen had been drawn 75 days after the patient was diagnosed as having aplastic crisis; the household contact was asymptomatic except for transient fever and nausea seven days before the onset of symptoms in the patient.

Of the 21 households for which sera were obtained from all members, 13 (61%) had an IgM-seropositive member other than the index patient with aplastic crisis, compared with none of 14 households of IgG-seronegative controls for whom complete sets of serum samples were available (P < .001). When households for which complete sets of serum samples were not available were included, results were similar: at least one member other than the patient with aplastic crisis was IgM seropositive in 12 (55%) of 22 households, compared with none of 22 households of the IgG-seronegative controls (P < .0001). For the IgG-seronegative household contacts of IgM-seropositive patients with aplastic crisis, the attack rates of 13 (37.1%) of 35 for those <20 years old and 9 (31.0%) of 29 for those ≥20 years old were similar (table 4).

In paired sera obtained from nine patients with aplastic crisis, there was no evidence of recent infection with parainfluenza virus types 1, 2, or 3; adenovirus; respiratory syncytial virus; *M. pneumoniae*; CMV; HSV types 1 or 2; Epstein-Barr virus; or varicella zoster virus.

**Outbreak of erythema infectiosum. Epidemiological studies.** Four-hundred and fifty cases of rash suggestive of EI were reported by Cuyahoga County school nurses from 1 January 1984 to 13 June 1984 (figure 1), after which most schools closed for summer vacation. Numerous physicians participating in these investigations reported that cases of EI were rarely observed in black children in this area. From 14 June 1984 to July 1985, no cases of EI were reported in Cuyahoga County, despite continued surveillance.

The median age of the 51 patients with EI from whom acute-phase serum samples were obtained was seven years (range, 1-17 years); 27 were male and 24 were female. The median age of the controls was ten years (range 1-16 years); 36 were male and 26 were female. Interviews with the parents of 28 IgM-seropositive patients with EI revealed that 27 (96%) of the patients had facial rash with "slapped cheek" appearance, 27 (96%) had rash on the trunk and/or limbs, 22 (79%) had a rash with a lacy reticular pattern, and 11 (39%) had transient recrudescences; the median duration of rash was 5.5 days (range, 2-21 days).

**Laboratory analysis.** In serum samples from 51 patients with EI, 54 controls, and 93 household con-
Table 3. Presence of B19-specific IgM in susceptible household contacts of IgM-seropositive patients with aplastic crisis or erythema infectiosum, Ohio, 1984.

<table>
<thead>
<tr>
<th>Age of household contact (years)</th>
<th>Aplastic crisis</th>
<th>Erythema infectiosum</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤4</td>
<td>2/5 (40.0)</td>
<td>9/13 (69.2)</td>
<td>11/18 (61.1)</td>
</tr>
<tr>
<td>5–9</td>
<td>5/11 (45.5)</td>
<td>8/8 (100.0)</td>
<td>13/19 (68.4)</td>
</tr>
<tr>
<td>10–14</td>
<td>3/8 (37.5)</td>
<td>6/9 (66.7)</td>
<td>9/17 (52.9)</td>
</tr>
<tr>
<td>15–19</td>
<td>3/11 (27.2)</td>
<td>3/5 (60.0)</td>
<td>6/16 (37.5)</td>
</tr>
<tr>
<td>≥20</td>
<td>9/29 (31.0)</td>
<td>11/20 (55.0)</td>
<td>20/49 (40.8)</td>
</tr>
</tbody>
</table>

NOTE. Susceptible contacts were those without B19-specific IgM in serum samples.

Table 4. Evidence of past infection with B19 in persons without B19-specific IgM from 105 households, Ohio, 1984.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>No. with B19-specific IgG/total no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤4</td>
<td>2/35 (5.7)</td>
</tr>
<tr>
<td>5–9</td>
<td>4/36 (11.0)</td>
</tr>
<tr>
<td>10–14</td>
<td>13/42 (31.0)</td>
</tr>
<tr>
<td>15–19</td>
<td>6/14 (42.8)</td>
</tr>
<tr>
<td>≥20</td>
<td>10/62 (16.1)</td>
</tr>
</tbody>
</table>

NOTE. The presence of B19-specific IgG in serum samples was considered to be evidence of past infection.

Characteristics of infection with B19. When susceptible household contacts of patients with EI or aplastic crisis were combined (table 3), the secondary attack rate was 59 (49.6%) of 119, and there was a significant decline in secondary attack rates with increasing age ($P = .027$). By comparison, when we excluded all IgM-seropositive persons and their household contacts (whose IgG seropositivity could have been the result of recent infection with B19), there was an increasing prevalence in IgG seropositivity as age increased, although this trend did not achieve statistical significance ($P = .136$; table 4).

When evidence of infection with B19 (presence of IgG or IgM) was examined with respect to sex in all patients with aplastic crisis or EI and their respective controls and household contacts, there were no significant differences in incidence between males and females <20 years old ($38 [29.2\%] \text{ of } 130$ and $23 [24.7\%] \text{ of } 93$), respectively, or between males and females ≥20 years old ($4 [12.9\%] \text{ of } 31$ and $12 [18.5\%] \text{ of } 65$). However, among household contacts with evidence of recent infection with B19 (excluding those with aplastic crisis), recent symptoms differed for household contacts of IgM-seropositive patients with sickle cell disease and aplastic crisis (all were black) and for household contacts of IgM-seropositive index patients with EI (all were white) when compared with their respective IgM-seronegative household contacts (table 5). Secondary cases of B19 infection among black contacts were less likely to have a rash (1 [6.3\%] of 16) than were secondary cases among white contacts (19 [52.7\%] of 36; $P = .002$), and there was a significant difference in rates of asymptomatic infection between white (6 [20.0\%] of 30) and black contacts (11 [68.8\%] of 16; $P = .003$). When black patients with secondary cases of B19 infection were compared with black control subjects,
### Table 5. Symptoms reported within 90 days by B19-specific IgM-seropositive and IgM-seronegative household contacts of patients with aplastic crisis or erythema infectiosum, Ohio, 1984.

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Household contacts of patients with aplastic crisis</th>
<th>Household contacts of patients with erythema infectiosum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgM seropositive (n = 16)</td>
<td>IgM seronegative (n = 41)</td>
</tr>
<tr>
<td>&quot;Fever&quot;</td>
<td>3 (19)</td>
<td>11 (27)</td>
</tr>
<tr>
<td>Fever (\geq 38.5^\circ C)</td>
<td>0 (0)</td>
<td>2 (5)</td>
</tr>
<tr>
<td>Headache</td>
<td>0 (0)</td>
<td>8 (20)</td>
</tr>
<tr>
<td>Running nose</td>
<td>2 (13)</td>
<td>9 (22)</td>
</tr>
<tr>
<td>Cough</td>
<td>1 (6)</td>
<td>7 (17)</td>
</tr>
<tr>
<td>Nausea</td>
<td>3 (19)</td>
<td>10 (24)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>2 (13)</td>
<td>4 (10)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1 (6)</td>
<td>5 (12)</td>
</tr>
<tr>
<td>Abdominal cramps</td>
<td>0 (0)</td>
<td>7 (17)</td>
</tr>
<tr>
<td>Muscle aches</td>
<td>0 (0)*</td>
<td>9 (22)</td>
</tr>
<tr>
<td>Joint pains</td>
<td>0 (0)</td>
<td>6 (15)</td>
</tr>
<tr>
<td>Chills</td>
<td>1 (6)</td>
<td>6 (15)</td>
</tr>
<tr>
<td>Any rash</td>
<td>1 (6)</td>
<td>4 (10)</td>
</tr>
<tr>
<td>Rash on arms, legs, and trunk</td>
<td>1 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Facial rash</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Lacy rash</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Recurring rash</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>No symptoms</td>
<td>11 (69)</td>
<td>22 (54)</td>
</tr>
</tbody>
</table>

**NOTE.** Excluding the household contacts of one patient with hereditary spherocytosis, all household contacts of patients with aplastic crisis were black, and those of patients with erythema infectiosum were white. All probabilities were calculated by Fisher's two-tailed exact test and compare IgM-seropositive and IgM-seronegative contacts.

* \(P \leq .05\).
† \(P \leq .001\).
‡ \(P \leq .01\).

No significant differences were found with respect to characteristics of classic EI (facial rash, rash on the trunk or limbs with reticular or lacy appearance, or occasional transient recrudescences), but significant differences between white patients with secondary cases of B19 infection and white control subjects were observed in all of these characteristic symptoms.

**Discussion**

This study of concurrent outbreaks of aplastic crisis and EI demonstrates the association of each of these clinical entities with a recent B19 infection. Examination of sera from patients with aplastic crisis and from controls revealed that only sera from patients had B19-specific IgM and DNA from B19. B19-like particles were also found by electron microscopy in acute-phase sera from five of the 24 patients. These sera specifically inhibited erythroid colony formation in vitro, a result supporting the hypothesis that aplastic crisis is due to cytotoxicity of B19 for erythroid progenitor cells in bone marrow [37, 44]. Examination of sera from patients with EI and from controls also revealed the presence of B19-specific IgM preferentially in sera from patients. This is the first report that describes close temporal and geographic association between outbreaks of these two syndromes. Interpretation of these findings favors a causal rather than coincidental relation because of (1) the consistency of case reports and other studies suggesting an association of parvovirus infection with aplastic crisis [1, 9–19] and EI [8, 18–23], (2) the biological plausibility of the hypothesis that infection with B19 can cause these two phenomena [44, 45], and (3) the strength of the associations demonstrated here by using serological data obtained from appropriately matched, susceptible controls.

The association of transient aplastic crisis in hemolytic anemia with a rash has been limited to a single previous case report [46]. Shortly after an outbreak of EI in London in 1983, two cases of aplastic crisis were described among patients with EI [46]. In the San Francisco outbreak, 15 % of patients with EI reported a rash, compared with 10 % of controls. These studies suggest a causal relationship between B19 infection and aplastic crisis, but further research is needed to confirm this association.
tic crisis were associated with B19 infection in a neighboring area, but these cases were not accompanied by a rash [13]. The classic descriptions of EI have been of an exanthem observed in populations of white Europeans and North Americans [7, 25]. The anecdotal reports we received regarding the relative absence of EI among black household contacts were not surprising, because other acute exanthems are more difficult to detect in black subjects [47], and a rash was a more common symptom of recent B19 infection among white contacts than among black contacts. The nonspecific symptoms reported in secondary cases suggest that B19 infection is likely to go unrecognized in a black patient who does not have underlying hemolytic anemia.

The estimated attack rate of aplastic crisis was higher for persons with SS disease than for persons with other sickle cell variants that are usually associated with milder hemolysis, longer survival of red cells, higher baseline levels of hemoglobin, and lower baseline reticulocyte counts [48]. During transient periods of erythroid aplasia, patients with SC disease or S-β-thalassemia and recently transfused patients with SS disease might not become as anemic as other patients with SS disease, and might be less likely to seek medical attention. The theory that some aplastic crises go undiagnosed is based on observations that some patients with hemolytic anemias have serological evidence of previous B19 infection without having a history of aplastic crisis [49, 50], as did five of our 33 controls.

Because B19 has only recently been propagated in culture [51], it is not yet clear whether B19 replication requires a helper virus. B19 packages equal numbers of single complementary DNA strands into separate particles [52], a property shared with those parvoviruses that cannot replicate autonomously [53, 54]. However, we found no serological evidence of recent infection with a possible helper virus (i.e., adenovirus or herpes group virus) in patients with aplastic crisis. The isolates retained strong inhibitory effects on erythroid colony formation after being heated to 56°C for 30 min, a result that would not be expected if a heat-sensitive helper virus were required for B19 replication; no other virus particles were revealed by electron microscopy [33]. These findings are consistent with electron microscopy in which no evidence could be found for a second morphologically distinct virus in bone marrow cells infected with B19 [44].

The increased number of erythroid progenitor cells in bone marrow observed in hereditary hemolytic anemias may facilitate B19 replication and account for the high concentrations of B19 observed in the acute phase of aplastic crisis. Concentrations of B19 DNA were at least several logarithmic orders of magnitude greater in the acute-phase sera of some patients with aplastic crisis than in sera from patients with EI (or their contacts) before and during the symptomatic period. These data suggest that it would be unusual for patients with normal hematologic constitution to have large quantities of B19 in sera. The intense B19 viremia reported after intranasal inoculation of normal adult volunteers [23] may reflect a response to a greater inoculum than that commonly experienced with natural B19 infection. Appreciable B19 viremia has rarely been observed under natural conditions in normal blood donors [55-57], but one possible explanation for the increased B19 replication is greater numbers of erythroid progenitor cells in bone marrow, perhaps caused by a recent hemolytic process or acute blood loss.

Although clinical studies have reported higher EI attack rates among girls than among boys [58, 59], and secondary cases of EI have been reported to be more common in mothers than in fathers [58], we found no significant gender-specific differences in rates of B19 infection. We did find that evidence of past B19 infection increased with age, and secondary B19 attack rates decreased with age, a result consistent with clinical observations that EI attack rates are higher in children and that occurrence of EI conveys long-term immunity [7, 58].

One possible risk factor associated with aplastic crisis was recent contact with a dog or cat, but living with a pet and having had recent contact with an unimmunized dog or cat were not significant risk factors. Other parvoviruses can infect canine and feline species [60], but it is not known whether cats or dogs can be infected with B19. Aplastic crises were not significantly associated with gender, presence of a rash, exposure to rashes in playmates or family, child care practices, or household population density.

The natural modes of B19 transmission are unknown. The most likely possibilities are transmission by fecal-oral, oral-oral, and/or respiratory routes, because B19 infection was very strongly associated with having a household contact who had evidence of recent B19 infection and was also associated (although not independently) with shared eating utensils. In most of the households of patients...
with aplastic crisis or EI, a member other than the patient had serological evidence of recent B19 infection, but household contacts of controls had no evidence of recent B19 infection. Although mutual contacts and factors other than those examined here could have been related to the introduction of B19 into any given household, the data presented here indicate that B19 commonly spreads to susceptible members of the household. The presence of B19 DNA in urine and throat gargle specimens suggests that the source of the virus may be urine and respiratory secretions and that attention to personal hygiene (e.g., frequent washing of hands) may prevent spread.

The spectrum of illness associated with B19 infection contains the minor rash associated with EI and more severe illnesses including aplastic crisis, acute polyarthropathy in adults [61, 62], and possibly fetal wastage [63, 64]; in these latter two sequelae, the role of B19 is unclear and needs to be defined by additional studies. Because aplastic crisis can be a life-threatening event [33], the data presented here suggest that patients with chronic hemolytic anemias should be closely observed and evaluated promptly for lethargy, malaise, headaches, abdominal pain, or other symptoms of aplastic crisis [3, 10, 11, 14] when outbreaks of EI or aplastic crisis occur, or when a family member has EI or an aplastic crisis.

References

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