SHORT REPORT

Aplastic anaemia associated with parvovirus B19 infection

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Parvovirus B19 involvement was investigated in 30 children with severe aplastic anaemia. Active or recent parvovirus B19 infection, as shown by B19 DNA viraemia, positive B19 specific IgM antibodies, or both, was diagnosed in six patients. There were no other plausible causes. We suggest that parvovirus B19 infection might be associated with severe aplastic anaemia.

Human parvovirus B19 has been associated with a broad spectrum of diseases including erythema infectiosum (EI) in children. One of the most common and serious complications of parvovirus B19 infection is transient aplastic crises in patients with chronic haemolytic anaemia such as sickle cell disease and hereditary spherocytosis. Pure red cell aplasia may also develop with a persistent infection of parvovirus B19 in immunocompromised individuals.¹ On the other hand, parvovirus B19 infection has been shown to be linked to idiopathic thrombocytopenic purpura and neutropenia.^{1,2} More recently, a case of severe aplastic anaemia (SAA) has been reported in a previously healthy boy without any underlying diseases, following asymptomatic infection with parvovirus B19.³ We studied the frequency of parvovirus B19 infection in children with SAA to explore the relation between parvovirus B19 infection and aplastic anaemia.

MATERIALS AND METHODS

Patients

A total of 30 children (18 boys and 12 girls) with SAA admitted to our hospital between April 1995 and December 1996 were studied. Median age was 6.8 years (range 1–14 years). The normal healthy control group was composed of 30 healthy children who came to our hospital for physical examination. Each of them was matched to a patient in the SAA group—that is, they were of the same age, from the same community, and were recruited almost at the same time.

The study conformed to the Helsinki declaration, and was approved by the local ethics committee. Informed consent was obtained from guardians of all children.

Samples

Thirty serum samples from the patients and 30 serum samples from the controls were tested for parvovirus B19 DNA and antibodies. Peripheral blood samples were collected from patients at the time of admission; serology was always done before administration of blood products.

PCR amplification

DNA was extracted from 50 µl of serum by lysis solution treatment.⁴ Nested polymerase chain reaction (PCR) was carried out for amplification of parvovirus B19 DNA using two primer sets as described previously.⁴ Appropriate precautions were taken during sample preparation and performance of the PCR to avoid cross contamination. There was no amplification

of DNA from cat parvovirus, adenovirus, cytomegalovirus, Epstein-Barr virus, herpes simplex virus, or hepatitis B virus (the specificity of the PCR test).

Antibody examinations of parvovirus B19

IgM and IgG antibodies for VP2, the major structural protein of parvovirus B19, were examined using ELISA with a commercially available kit (IBL, Hamburg, Germany).

RESULTS

We examined the presence of parvovirus B19 DNA and antibodies in cases of SAA by PCR and ELISA. Six of the 30 patients were positive for parvovirus B19 DNA in the sera. Four of 30 (13.3%) had B19 IgM antibodies. Five of 30 (16.7%) were B19 IgG seropositive. No parvovirus B19 DNA or IgM antibodies were detected in normal children. The occurrence of parvovirus B19 DNA in the SAA group was significantly higher than in normal controls ($p = 0.02372$, Fisher's exact test). Table 1 shows the virological and laboratory data of these six patients. Virological examination showed that no patient was infected with Epstein-Barr virus, cytomegalovirus, or hepatitis A, B, or C viruses.

Of the six parvovirus B19 DNA positive patients, two had a history of recent EI, two and three weeks before the onset of SAA; the other four cases had asymptomatic B19 infection except for haematological disturbance. Two of the six died from haemorrhage; four had achieved complete remission following treatment with combination therapy consisting of horse antilymphocyte globulin (10–15 mg/kg/day for five days), cyclosporin A (3–6 mg/kg/day for 3–6 months), and intravenous immunoglobulin (400 mg/kg/day for five days).

DISCUSSION

Aplastic anaemia is a bone marrow haemopoietic failure induced by a variety of causes, and its aetiology remains unclear thus far. It has been recognised that aplastic anaemia is associated with certain chemicals, drugs, radiation, and virus infections. Epstein-Barr virus and non-A, non-B, or non-C hepatitis virus precede aplastic anaemia in some patients with this disorder. However, little is known about the causal relation between parvovirus B19 infection and aplastic anaemia. In a recent report, a 14 year old boy with no obvious underlying disease who developed SAA following parvovirus B19 infection was described.³ In order to investigate the relation between parvovirus B19 infection and SAA, we studied the occurrence of parvovirus B19 DNA and antibodies in 30 cases of SAA, and found six cases associated with active or recent parvovirus B19 infection. The absence of detectable antibodies with positive B19 DNA in patient 6 may have occurred because of immunocompromise. There were no other

Abbreviations: EI, erythema infectiosum; PCR, polymerase chain reaction; SAA, severe aplastic anaemia

Table 1 Clinical, laboratory, and virological data of six patients with SAA associated with parvovirus B19 infection

Patient no.	Age (y)	Sex	Chronology of events	History of EI	Parvovirus B19		
					DNA	IgM	IgG
1	1	M	Apr 1995	—	+	+	+
2	5	F	Jun 1995	+	+	+	+
3	4	M	Aug 1995	—	+	—	+
4	8	M	Feb 1996	+	+	+	+
5	12	M	May 1996	—	+	+	+
6	2	F	Sep 1996	—	+	—	—

plausible causes. Our study suggests that parvovirus B19 infection might be associated with childhood SAA. This virus should therefore be considered as a possible aetiological agent in some children with SAA.

The role of parvovirus B19 infection in the pathogenesis of aplastic anaemia is unclear. Two hypotheses can be advanced, the first of which involves the direct effect of parvovirus B19. It has been shown that the cellular receptor for this virus is an antigen of the group blood P, which is present not only on erythrocytes and erythroblasts, but also on megakaryocytes and fetal liver cells.¹ Therefore, parvovirus B19 infection can result in transient aplastic crises, congenital or acquired pure red cell aplasia, and idiopathic thrombocytopenic purpura, for example. Experimental infections with parvovirus B19 in normal volunteers showed that not only erythroid production, but also myeloid and platelet production were affected. Moreover, granulocytopenia and thrombocytopenia, which can occur with acute parvovirus B19 infection, are also probably a result of the cytotoxic effect of the NS1 protein of the virus.⁵ The above data suggest that all the three precursor cell lines in bone marrow might become the target cells of parvovirus B19 infection. The second hypothesis is based on immunological mediation. In virus associated haemophagocytic syndrome with acute parvovirus B19 infection, raised cytokines such as interferon γ would impair regulation of the phagocytic system, resulting in pancytopenia and/or decreased haematopoiesis.^{3,6} The recovery of haematopoietic function after immunosuppressive therapy is the strongest argument for this hypothesis. The precise mechanisms involved in the pathogenesis of parvovirus B19 associated aplastic anaemia need to be studied further.

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