Various Associations of Human Parvovirus B19 Infection

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Abstract

A variety of diseases encountered in association with human parvovirus 1319 infection seen at Paediatric clinic during 6 months is presented and their relation to parvovirus B19 is discussed. We conclude that investigation of paravivirus 819 in variable diseases by using the newly developed methods of molecular biology will enlighten many etiopathogenetic mechanisms (JPMA 46:235, 1996).

Introduction

Human parvovirus B19 (B 19) was accidentally discovered in 1975 in human serum while evaluating screening tests for hepatitis B virus. The newly found virus was named B19 as a reference to the batch in which it was initially found1,2. Subsequent studies of its effects in humans have identified this virus as being able to induce aplastic crises in sickle cell disease and to be the causative agent of erythema infectiosum. The virus is known to induce aplastic crises in patients with hereditary spherocytosis and other chronic haemolytic anemias. The most common manifestation of human parvovirus infection, however, is erythema infectiosum (fifth disease). Many clinical characteristics of B 19 infections have been defined since 1975, especially during the last 10 years many well-defined clinical syndromes have been added to the list of diseases caused by this virus, but others are still unclear3,4. B 19 is ubiquitous and may occur year-round, but outbreaks of erythema infectiosum (EI) are most common in spring. Most infections occur among school-age children. Prevalence of IgG antibodies against the virus is high (30% to 60%) in adulthood5. B 19 DNA is found in respiratory secretion of viremic patients and outbreaks of erythema infectiosum in schools may be prolonged over months, suggesting close contact rather than aerosol transmission. Patient-to-patient transmission of B 19 associated aplastic crisis has occurred. Vertical and parenteral transmission may occur6-8. Although, it is rare and the classical clinical picture is arthropathy in adults, erythema infectiosum in children and asymptomatic infection in all age groups. The diseases caused by this virus are divided into three main categories: 1) diseases found among normal hosts (asymptomatic disease, EI, arthropathy, hydrops fetalis); 2) Haematologic diseases (aplastic crisis, chronic anaemia, idiopathic thrombocytopenic purpura-Itp, transient erythroblastopenia of childhood, Diamond-Blackfan anaemia); 3) Heterogenous group of diseases, in which the etiologic role of parvovirus is less clear and sometimes putative (neurologic disease, rheumatologic disease, vasculitic and myocarditic syndromes). In particular, EI, hydrops fetalis and the hematologic diseases may be paediatric concern1,7. Here we studied the diseases associated with serologically proven B19 infection during a six month period at a paediatric clinic.

Materials and Methods

The diagnosis of the viral infection was based mainly on clinical and serological criteria (detection of specific IgM, then IgG, antibodies by ELISA technique) Identification of specific IgM and IgG antibodies to B i 9 is a reliable method in the diagnosis of B19 infection9,10. Acute 819 infection was considered to be present if the anti-B 19 IgM index was >1.2 (sensitivity 89%, specificity 98%)11.
Specific IgM and IgG antibodies to B19 were investigated by using ELISA method (IDEIA TM-Parvovirus 819 IgM/IgG-DAKO AIS, Denmark) in patients referred to the clinic between March-October 1993 with different complaints, in whom one or more of the following laboratory findings were identified: anaemia, reticulocytopenia, thrombocytopenia and erythroid hypoplasia or aplasia on bone marrow aspiration. Patients in whom specific IgM antibodies (serologically suggesting acute or just convalesced B19 infection) were identified and entered the study. Test principle: The reaction wells were specifically coated for IgG and IgM test with different recombinant surface proteins of the B19. IgG antibodies were detected by the VP1 protein, whereas the IgM immune response was measured by the VP2 protein.

The specific proteins, which are immobilized on the testplate, bind to the corresponding antibodies in the sample. Plates were incubated at room temperature for 60 minutes. The complex reacts with the enzyme conjugate and after adding the substrate a yellow colour is produced by enzymatical cleavage. Unbound reactants are removed by washings. The resultant colour change was quantified by spectrophotometry at a wavelength of 450 nm. Optical density was directly proportional to the amount of antigen-specific IgM or IgG present in the sample. To report test results in a numerical form relative to the cut-off, the patients’ optical density values were divided by the mean of the cut-off calibrator absorbance values. The quotient was referred to as the index result of every case. In order to eliminate false positive results caused by rheumatoid factors aRF-Absorbent was used which is included in the kit. Patient sera were mixed with this solution and then assayed. After B19-specific IgM antibodies were demonstrated, haematologic studies, including haemography, reticulocyte count and (when indicated) bone marrow aspiration were conducted. Specific laboratory investigations necessitated by the clinical condition of each patient were carried out. In all but one patient, serological tests for B19 were repeated in the succeeding 8-16 weeks after the first investigation. The possibility of acute infection due to the following agents was excluded by the indicated serological analyses: Toxoplasma and rubella virus (microparticle enzyme-immunoassay, Abbott, Chicago, USA); cytomegalovirus, herpes simplex virus and measles virus (EIA, Enzignost, Bebring, Marburg, Germany); and Epstein-Barr virus (indirect immunofluorescence IgM anti-VCA, Gull Laboratories, Salt Lake City).

Results

Of 9 patients included in the study, 5 were girls and 4 boys. The age range was 1 month to 8 years. Complaints on referral, physical examination and laboratory investigations are summarized in Table I.
Anaemia was identified in six cases, reticulocytopenia in one and thrombocytopenia (ITP) in three cases. Variable degrees of erythroid hypo-aplasia was seen on bone marrow aspiration in all five patients excluding patient 6 and 7 in whom idiopathic thrombocytopenic purpura was diagnosed and patient 5 and 9 on whom bone marrow aspiration was not performed. During initial investigations, all nine patients had specific IgM antibodies to B19. IgM antibodies disappeared in six patients, both IgM and IgG positivity persisted in one patient who had been diagnosed as leukocytoclastic vasculitis. In patient 8 with pure red cell anemia (possibly chronically infected case), IgG antibody production were absent but IgM antibodies had become negative and in patient 9 with hydrops fetalis, IgM antibodies persisted for 6 months, but IgG antibodies were negative. The diagnoses in our patients were as follows: Celiac disease+iron deficiency, megaloblastic anemia due to vitamin B12 deficiency, acute lymphoblastic leukemia (ALL), leukocytoclastic vasculitis + iron deficiency, hemolytic anemia, ITP, fetal hydrops and pure red cell anemia. Serological tests for B19 were performed while the signs and symptoms of disease appeared in the patients with ALL and 1W, after the disease signs and symptoms had established in the others (Table II).
Discussion

Although the major target cell population of B19 in human is erythroid cell lines, it can invade and cause a variety of infections in different types of cells. It is reported that B19 uses erythrocyte P antigen (globoside) as a receptor for entrance to the cell and this antigen is expressed in cells other than erythroid cells lines \(^6,8,12\). Rarely individuals lack P antigen (The P negative phenotypes: PiK and p) and, remarkably, are not susceptible to B19 infection \(^1,8,12\). Knowledge of a parvovirus receptor has implications for the understanding of the pathogenesis of B19 infection and the possible use of parvoviruses in gene therapy \(^1\). In all of the diseases defined in relation with B19 infection until now, patients' immunological response to the virus and haematological status played a major role in the development of the symptoms of clinical disease. In particular, it is interesting that the clinical disease observed in the patients in whom the humoral immune system, which builds up the strongest defence mechanism against B19 cannot provide an effective viral neutralization both qualitatively and quantitatively \(^13-15\). Varying clinical manifestations are due to a combination of the erythroid aplasia and the immunological status of the infected host. In normal host; infection results in a self-limiting subclinical erythroid aplasia, followed by a presumably immunologically mediated rash or arthralgia \(^1\). Since the subjects with unstable haematological processes such as iron deficiency and vitamin B12 deficiency anaemia and haemolytic anaemia with hyperplasia presents a favourable environment for B19 infection, it is not a surprise to encounter B19 infection in these patients. Among the patients with iron deficiency anaemia, no etiological association has been defined between Celiac disease and B19. However, in our patients with vasculitis in whom iron deficiency developed due to chronic blood loss, B19 might have an etiopathological role as mentioned above. It is possible that an underlying immune disorder predisposed our patients to both chronic B19 infection and vasculitis. Various immune abnormalities have been reported in patients with systemic vasculitis \(^16\). B19 associated vasculitis brought new insights to the spectrum of this virus \(^17-19\). The foetus has a rapidly expanding red cell volume, diminished red cell survival (45-70 days) and decreased capacity for clearing infections.

Table II. Serological tests and the diagnosis of patients.

<table>
<thead>
<tr>
<th>B19 Serology</th>
<th>Diagnosis</th>
<th>Time of the serological test for B19*</th>
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<tr>
<td>Initial</td>
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<td>IgM</td>
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<td>Celiac disease+</td>
<td>Iron deficiency</td>
<td>After</td>
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<tr>
<td>ALL</td>
<td>Iron deficiency</td>
<td>Concomitant</td>
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<tr>
<td>Leucocytosclastic vasculitis+</td>
<td>Vitamin B12 deficiency</td>
<td>After</td>
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<tr>
<td>Megaloblastic anaemia+</td>
<td>Hemolytic anaemia**</td>
<td>After</td>
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<tr>
<td>Pure red cell aplasia</td>
<td>Pure red cell aplasia</td>
<td>After</td>
</tr>
<tr>
<td>Hydrops fetalis</td>
<td>Hydrops fetalis</td>
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Consequently, the fetus has greater susceptibility to the ill effects of certain viruses, e.g., B 191. It has been postulated that fetal infections represent the clinical manifestations of the first phase of B 19 infection. Among pregnant women with a confirmed primary B19 infection, it is believed that the risk of an abnormal outcome is approximately 5-10% and the risk is highest if the infection occurs during the first 20 weeks of gestation. Fetal parvovirus infection may manifest itself as non-immunologic hydrops fetalis, fetal or congenital anaemia, abortion, stillbirth, or as an asymptomatic self-limiting episode. B19 infects the erythroid progenitors of the fetus leading to severe anaemia and hypoxia. Anaemia may provoke high output cardiac failure, causing fluid to accumulate in the body cavities, resulting in generalized edema and possible death. Because of decreased capacity for clearing infections, in our patient 9 with hydrops fetalis, (probably chronically infected patients), IgM antibodies persisted for a minimum of 6 months, but IgG antibodies appeared only after the 7th month. The persistence of specific IgM antibody for a minimum of 2 years and possibly 4 years has been reported²,³,¹¹. B19 infection can cause thrombocytopenia ²⁰-²². Longevity of B19 in megakaryoblastic cell cultures in the presence of erythropoietin suggested that the virus can infect the megakaryocytes²³. In addition, B 19 is proposed to slow the megakaryocytic colony formation⁹. However, it is not clear whether viral invasion has a direct suppressive effect on the megakaryocytes or the immune response against the virus leads to the development of thrombocytopenia. Identification of specific IgM antibodies while the findings of ITP developed in our patient suggests B 19 can be an etiological agent in ITP. Since the thrombocytes also carry the erythrocyte P antigen, it can be suggested that the thrombocytes altered by the viral invasion can be exposed to immune attack or the thrombocytic destruction occurs when immune response against the virus is directed to P antigen on the thrombocytes by anti-idiotypic mechanism. On the other hand, remegakaryocytic cell line which is activated in the bone marrow because of thrombocytopenia caused by a different mechanism and entered mitosis with increased numbers of the young elements, will provide a favourable environment for B19 infection which needs the S phase factors; therefore, 1319 infection in ITP may be an incidental association. Development of ALL after a case of 1319 infection leading to bone marrow hemolysis has been reported²⁴. In our case of ALL, specific IgM antibodies to B19 were identified during the period when the findings of disease developed; and it was shown by serial bone marrow aspirations that the bone marrow findings of ALL progressed slowly. Though it is somewhat early, it may be discussed that B19 participates directly or indirectly in the development of some of the hematological clonal diseases. Alternatively, it is possible that some leukemia cells can be a favourable target for B19. Sometimes B 19 provokes a decrease in the levels of CD4 and CD8 positive T-lymphocytes, 8-16 days after inoculation. There is usually no change in the number or morphology of monocytic, basophilic or eosinophilic granulocytes and only infrequently, atypical lymphocytes are observed¹. The relation of B19 infection and pure red cell anaemia in subjects with inadequate immune response to B 19 has been reported¹⁴. Many investigators have also reported cases in which patients were unable to mount an efficient antibody response due to an underlying immuno-deficiency. In our case, though an initial IgM antibody response had been developed to 1319, an IgG response was not detected later. It must be remembered that investigation of the viral DNA will contribute to the explanation of this process. These findings may suggest that IgG antibodies playing a major role in the viral neutralization especially in the long-term, cannot develop and the infection can not be controlled. Chronic B19 infection is associated with defective or delayed antibody formation to viral antigens. In some chronically infected patients, IgG antibody production is absent. Other such patients might suffer quantitative and qualitative IgG defects¹. Some immunocompetent patients have been reported not to mount a detectable IgM response⁵ and 15-20% do not produce specific IgG²⁵. Alternatively, negative or equivocal serology could indicate immunodeficiency. Equivocal IgG and IgM titers is a phenomenon typical for persistent infections in patients with immunodeficiency²⁵. Several patients have been described with chronic B19 infection accompanying subtle forms of immune dysfunction, characterized
by qualitatively abnormal immune responses rather than absolute immunodeficiency. Identification of specific IgM and IgG antibodies to B 19 is a rather reliable method in the diagnosis of B19 infection. Very often, search for B19 DNA alone, or in combination for specific antibodies, will prove beneficial. However, when the investigations of viral DNA in the body fluids and at the tissue level is combined with the results of the serological tests, the evaluation can be much more significant. Particularly in cases involving those suffering from like persistent congenital red cell aplasia, the clinician should, when looking for a possible B19 infection, always continue the search using the PCR technique, even if the antibody tests are negative. Next step will be to reveal that the identified virus has caused cellular changes (apoptosis etc.) which led to the disease process, because, B 19 probably causes the pathologic changes by varying types of infection (productive, persistent, etc.) in different cells. 819, whose relation with many diseases has been defined at present, seems to keep on being a subject of interest. In this context, it will be important to differentiate the coincidental associations from the etiopathogenetic relations.

References