Parvovirus B19 May Have a Role in the Pathogenesis of Juvenile Idiopathic Arthritis

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ABSTRACT. Objective. To determine the prevalence of human parvovirus B19 infection in patients with juvenile idiopathic arthritis (JIA) by detection of specific IgM, IgG, and viral DNA.

Methods. Serum samples of 50 patients with diagnosis of JIA and 39 healthy controls were analyzed by ELISA to detect IgG and IgM anti-B19-specific antibodies. The parvovirus B19 genome was detected by nested polymerase chain reaction (PCR). The average age of the patients was 9.6 years (2–14 yrs); 30 were female (60%) and 20 male (40%). The definitive diagnoses of these patients corresponded to 19 systemic forms (38%), 11 to the oligoarticular variety (22%) and 20 to the polyarticular (40%). The average age of the control group was 7.8 years (2–16 yrs); the distribution by sex was 25 females (64%) and 14 males (36%).

Results. IgM against parvovirus B19 was detected in 20% of the cases (10 patients) and B19 DNA genome by PCR in 48% (24 patients); in 10% of the cases (5 patients), both markers were detected. IgG was found in 32% (16 patients). In the control group neither IgM nor the viral genome was detected. However, 43.5% of the controls (17/39) had IgG against parvovirus B19, indicating past infection by the virus.

Conclusion. Our study confirms recent observations regarding a high prevalence of viral DNA in JIA patients and a possible role of this viral infection in JIA pathogenesis. (First Release May 1 2007; J Rheumatol 2007;34:1336–40)

Key Indexing Terms:
PARVOVIRUS B19 JUVENILE IDIOPATHIC ARTHRITIS

Juvenile idiopathic arthritis (JIA) is the most common rheumatoid disease in children[1]. There are several different types of juvenile arthritis and the prognosis varies according to each particular form of the disease[2-5].

A few viral infections are known to develop with articular symptoms of limited course; however, in some cases they may present a prolonged progression similar to JIA. Viral arthritis has been reported with a number of viruses including varicella-zoster, coxsackie, Epstein-Barr, herpes simplex, cytomegalovirus, rubella, hepatitis B, and parvovirus B19[6-10]. Parvovirus B19 is a single-stranded DNA virus; the particle is composed of 2 capsid proteins, VP1 and VP2, and a nonstructural protein, NS1[11-13].

Parvovirus B19 has been associated with a wide range of disease manifestations, whose severity depends on the immunological and hematological status of the host. In normal, immunocompetent individuals, the most common manifestation of infection with the virus is erythema infectiosum (fifth disease). In individuals with underlying hematological disorders such as sickle cell disease, parvovirus B19 infection results in aplastic crisis. Infection during pregnancy may cause fetal hydrops and spontaneous abortion. Anemia, red cell aplasia, and arthropathies may occur in immunocompromised patients with chronic parvovirus B19 infection. Acute B19 infection is thought to confer a protective, lifelong immunity. However, persistent B19 infection has been reported to occur not only in immunocompromised patients, who may develop chronic anemia and thrombopenia, but also in immunocompetent individuals, who may develop chronic arthropathy[14].

During the acute phase of B19 infection, a high-titer viremia (> 10^{13} genome equivalent copies/ml) may be observed; while during the convalescence or in a course of chronic infection the viral titer decreases and viral DNA may be found at lower titers, detectable only by polymerase chain reaction (PCR)[15]. Specific IgM antibodies develop during the second week after infection and may be detectable for 4–6 months and sometimes longer. Specific IgM can therefore be present simultaneously with B19 DNA, although in some cases either IgM or DNA is detected. Specific IgG antibodies appear several days after IgM and persist for years; their presence is generally a sign of past infection[16].

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Several clinical studies have linked evidence of earlier B19 infection and B19 viral persistence with chronic arthritis including adult rheumatoid arthritis (RA) and JIA[17-22]. These observations suggest that, at least in some cases of JIA, parvovirus infection may have a role in the cause of this disease.

Our aim was to study the relationship between parvovirus B19 infection and the diverse forms of JIA with a prolonged course in children.

MATERIALS AND METHODS

Patients. Serum samples of 50 patients with JIA, who were treated at the Immunology Unit of the Luis Calvo Mackenna Hospital in Santiago, Chile, were included in our study. All were consecutive inpatients of a pediatric immunology ward between 1993 and October 1996. The clinical diagnosis was defined according to the juvenile arthritis criteria of the American Rheumatism Association (ARA)[23] and the European League Against Rheumatism (EULAR)[24]. Independently, patients were later reclassified according to the criteria developed by the International League of Associations for Rheumatology (ILAR)[25]. The majority of the patients were classified in the same groups currently defined for JIA. Although HLA-B27 was not studied in pauciarticular (currently oligoarticular) patients, all except one were less than 8 years old.

This group was composed of 30 females (60%) and 20 males (40%), mean age 9.6 years (range 6 mo to 12 yrs). With respect to their clinical diagnosis, the children were classified into 3 subgroups (Table 2): polyarticular [n = 20, 14 rheumatoid factor (RF)-negative and 6 RF-positive], systemic arthritis (n = 19), and pauciarticular (n = 11). The average disease duration previous to the study was 4.7 years (1–12 yrs). The criteria for active RA were based on the recommendations established by the American College of Rheumatology[26].

All the patients had complete blood counts, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), antinuclear antibodies (ANA), RF, and radiological studies of the affected articulations.

Our study was carried out with the consent of all patients through their parents and received approval from the ethics committee of the Faculty of Medicine, University of Chile.

Controls. Serum samples were obtained from 39 children who were treated in the otolaryngology ward in the same hospital, between 1993 and October 1996. This group was composed of 25 females (65%) and 14 males (36%), mean age 7.8 years (range 2–16 yrs). At the moment of blood sampling, none of the children or adolescents presented any acute or chronic illness (e.g., erythema infectiosum, anemia, arthritis, etc.) related to parvovirus B19 infection.

Detection of parvovirus B19-specific antibodies. Serum samples were kept frozen at –80°C until use. Antibodies IgG and IgM specific against VP1 protein were determined using an enzyme linked immunosorbent assay (ELISA; Focus Technologies, Dallas, TX, USA). Testing was performed according to the manufacturer's instructions.

Nested PCR for B19 virus DNA. All the sera samples were screened initially by nested PCR for a conserved region of the NS1 gene[27], using 2 different reactions. DNA was extracted from samples of 40 μl of serum by phenolchloroform extraction. A 10 μl sample of DNA extract was added to 40 μl of master mix containing 2.5 mM $MgCl_2$, Buffer 10X, 100 mM NaCl, 1.5 mM dNTPs, 37.5 pM primers 1 and 6, and 2.5 U of Taq Polymerase. The second reaction utilized the same mixture, but with primers 2 and 5, instead of primers 1 and 6[27]. Primers 1, 2, 5, and 6 correspond to nucleotides 1399-1422, 1498-1525, 1576-1600, and 1659-1682, respectively, of the NS1 gene of B19 virus genomic DNA[12]. For both reactions, PCR amplification was performed as follows: 2 min incubation at 94°C; 35 cycles of 30 s at 94°C, 30 s at 55°C, and 1 min at 72°C; and a final incubation for 5 min at 72°C. The second PCR was run for 25 cycles. PCR using primers 1 and 6 yielded a product of 284 bp in the first round and PCR using primers 2 and 5 yielded a product of 103 bp in the second round[27]. Ten microliters of the final product were analyzed in ethidium bromide-stained 1.5% agarose gels.

The B19 DNA-positive samples were studied further with nested PCR for 2 other B19 genomic regions[28]. The outer primer pair VP2ofwd and VP2orev and the inner primers pair VP2ifwd and VP2irev were used for the VP2 region and the outer primer pair NSofwd and NSorev and the inner primer pair NSifwd and NSirev were used to amplify a different region of the NS gene. The same PCR were used as controls for doubtful cases.

Statistical analysis. Age differences were analyzed by the Student t-test and sex differences were determined by z-test. Continuous variables were compared using the Mann–Whitney test and the categorical data were analyzed using a chi-square test. All comparisons were 2-sided and a p value $\leq$ 0.05 was considered significant.

RESULTS

Detection of parvovirus B19-specific antibodies. Initial testing of all sera for the presence of parvovirus B19-specific humoral responses revealed that 16 (32%) of 50 patients with JIA and 17 (43.6%) of 39 controls demonstrated IgG against the VP1 protein, indicating previous infection in both groups. IgG antibodies against the VP1 structural protein were detected in these patients irrespective of the manifestation of the rheumatic disease (Table 1).

Specific IgM reactivity against VP1 protein was detectable in 10 (20%) of the 50 JIA patients' sera and none of the 39 control sera ($p < 0.01$; Table 1).

Detection of viral genome. Samples that gave the appropriately sized PCR products (284 bp for the primary product and 103 bp for the nested product) were considered to be positive. Parvovirus B19 NS1 DNA was detected in 24/50 of the patients (48%), whereas in the control group, all PCR were negative ($p < 0.01$; Table 1). Positive samples were tested a second time and were only classified as positive when the results of the first test could be confirmed independently. Among the first NS1-positive sera, all the samples gave the expected positive results in B19 VP2- and the second NS1-PCR (using B19 primers as described[28]).

Additionally, 7 of 24 samples of JIA patients positive for PCR were sequenced using primers designed for the region VP1/VP227. DNA sequence analysis revealed typical sequences of B19 genomes and the samples were slightly divergent (0 to 2.9%, data not shown).

Parvovirus B19 genome was detected in association with all JIA manifestations (Table 2): systemic, 10 of 19 (52.6%); polyarticular, 9 of 20 (45%); and pauciarticular, 5 of 11 (45.5%). From these 24 positive samples for B19 DNA, 7

Table 1. Parvovirus B19 infection in patients with JIA and control group.

	JIA	Control	p
Cases, no.	50	39	
Age, yrs, median*	9.9	7.0	NS
Sex, M/F**	20/30	14/25	NS
IgG† (%)	16 (32)	17 (43.6)	NS
IgM† (%)	10 (20)	0	0.01
Viral DNA† (%)	24 (48)	0	0.01

* t test, ** z-test, † chi-square test or Fisher's exact test. NS: not significant.

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Table 2. Clinical and viral markers of patients with JIA according to the presence of parvovirus B19 DNA.

JIA	Total Cases		Systemic JIA		Polyarticular JIA		Pauciarticular JIA	
Markers	PCR+	PCR–	PCR+	PCR–	PCR+	PCR–	PCR+	PCR–
	24	26	10	9	9	11	5	6
IgM-positive	5	5	1	2	3	2	1	1
IgG-positive	7	9	2	3	2	3	3	3
p†	NS		NS		NS		NS	
JIA active at the last review	16/24	7/26	8/10	2/9	7/9	4/11	1/5	1/6
p†	0.05		0.02		NS		NS	
Time of clinical activity, median, mo	36	36	54	30	36	36	36	41
p†	NS		NS (0.1786)		NS		NS	

† Chi-square test, Mann-Whitney test. NS: not significant. PCR: polymerase chain reaction; JIA: juvenile idiopathic arthritis. NS: not significant.

were positive for IgG and 5 for IgM. Further, in 5 patients with no viral B19 DNA, IgM was detected (Table 2). Considering the presence of B19 DNA and/or the presence of B19-specific IgM as a diagnostic test for B19-associated diseases, 29 of the 50 patients with JIA (58%) were positive for B19 disease: 5 had both B19 DNA and IgM (10%), 19 had B19 DNA without IgM (38%), and 5 had IgM without B19 DNA (10%) ($p = 0.58$; Table 3).

Table 2 shows some characteristics of patients with positive or negative parvovirus B19 PCR. The characters included are: type of JIA, time of evolution, age, and the presence of specific IgM and IgG anti-B19. Although the total time since diagnosis was not different among the children with or without viral DNA detection, the presence of viral DNA was more frequent in the cases of JIA that showed clinical activity in the last review ($p < 0.05$). In these patients there were no differences in IgM and IgG detection.

There was a significant difference in the genome detection in those patients with systemic JIA that at the time of the analysis had signs of clinical activity (8/10, 80%), in contrast to those who were asymptomatic (2/9, 22%; $p < 0.02$). A similar but not statistically significant tendency was detected in patients with polyarticular JIA; in these 7/9 patients (77.7%) with signs of clinical activity DNA were detected in comparison with the patients with no activity (4/11, 36%). In patients with the pauciarticular type, no differences were observed in the IgM and IgG detection or in the viral genome among the patients regardless of their clinical activity.

The immunologic markers of JIA, such as ANA and RF, were related neither to the presence of IgM nor viral DNA, nor to the clinical activity of the disease (data not shown).

DISCUSSION

Our study showed that the previous infection rate by parvovirus B19, as detected by specific IgG, was similar in children with JIA and the control group (32% and 43%, respectively). It is important to mention that of the different types of JIA, the pauciarticular form was observed in a higher number of children with B19-specific IgG given by previous infection (54%). Lehmann, *et al* found that 62% of the children with JIA had specific B19 IgG[22]. However, their analysis included antibodies against all the structural proteins of the virus, whereas we detected only those antibodies directed to VP1 protein. These results indicate that previous infection by parvovirus B19 in patients with JIA and normal controls is similar. This antibody prevalence in both groups is in a range that would be expected in children, since parvovirus B19 infection is known as one of the classic diseases of childhood[11,13].

Parvovirus B19-specific IgM was detected in 20% of the patients with JIA (10/50) and it was not possible to assign it to any specific form of JIA. The presence of specific IgM antibodies in our study group matches the results reported by Kishore, *et al*, who found that of 69 patients with JIA, 27.5% had IgM and 13% were positive for IgM and IgG[19]. The serum samples used in our study were collected when the patients had already had a previous expression of the disease,

Table 3. Presence of B19 DNA and/or presence of B19-specific IgM in 50 positive serum samples of patients with JIA.

B19 DNA Sera*	B19 IgM + Sera		B19 IgM – Sera		Total Sera	
	N	%	N	%	N	%
B19 DNA +	5	10	19	38.0	24	48.0
B19 DNA –	5	10	21	42.0	26	52.0
Total	10	20.0	40	80.0	50	100

* $p = 0.58$, Fisher test.

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and therefore the presence of IgM may not be associated as a marker of acute primary infection. One explanation of the presence of IgM in some patients might be a reactivation of the virus as a product of a persistent infection, or a new viral infection.

The persistence of parvovirus B19 in children with JIA has not been fully studied. Most of the work has been focused on adults with RA. In these individuals the virus has been found in synovial tissues, T and B lymphocytes, and dendritic cells[29-32]. Kerr, *et al*[33] detected viremia in 7 out of 53 patients 3–5 years after the infection. Our study confirmed the presence of viral DNA in 48% of the patients with JIA. Lehmann, *et al* reported the presence of viral DNA in 38% of the patients with this pathology[22]. In that study, the control group showed viremia in 7% of the cases. In contrast, we did not detect viremia in the control group patients. The conclusion of these observations is that patients with JIA show a higher viremia than healthy children, in agreement with the possibility that B19 may play a role in the pathogenesis of JIA.

Although the inability to develop an efficient neutralizing immune response is observed mainly in immunosuppressed individuals, healthy individuals may fail to eliminate the virus, thus leading to persistent infection and the possible occurrence of chronic diseases such as chronic anemia or arthropathies[11]. In some cases of patients under immunosuppressive drug treatment such as in children with transplants, persistent viremia is often observed[34,35].

The detection of parvovirus DNA in 48% of the patients with JIA indicates that they have developed persistent B19 infection and are incapable of eliminating the virus. This may be due to a different mechanism, for example, an inadequate immune response against the viral capsid proteins[36]. It cannot be ruled out that in some patients, the capacity of the immune system to eliminate the virus may be restricted by the immunosuppressive treatment with steroid drugs. In our study, a high percentage of patients had received treatment with immunosuppressive drugs such as methotrexate or oral steroids over long periods. These treatments produce an alteration of the immune response and probably induce a virus reactivation.

The presence of viral DNA was more frequent in children with active JIA than in children without clinical activity in the last review. With respect to the different groups of patients (pauciarticular, polyarticular, and systemic), it may be important that viral DNA could be detected in the majority of the children with systemic active JIA. Thus, the correlation between parvovirus B19 DNA and clinical activity in children with the systemic form of JIA is very interesting.

The detection of parvovirus DNA in sera obtained years after primary infection may contribute to our understanding of the generation of long-lasting rheumatoid complaints during childhood. An important question is if parvovirus B19 is an agent associated with pathogenesis of JIA or if it only acts as an agent that triggers the clinical manifestations of the disease.

Our work demonstrated a higher frequency of parvovirus B19 infection markers (DNA and IgM) in children with JIA than in the control group, and also that active JIA correlated with the persistence of parvovirus DNA but not with IgG antibodies to parvovirus. Our study confirms recent observations regarding a high prevalence of viral DNA in patients with JIA and a possible role of this infection in the pathogenesis of JIA.

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