Incidence and Clinical Significance of Parvovirus B19 Infection in Patients with Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** To determine the prevalence and clinical significance of human parvovirus B19 (B19) infection in patients with rheumatoid arthritis (RA).

Methods. One hundred patients with RA and 94 apparently healthy blood donor controls were enrolled for study. Plasma samples of patients and controls were examined for the presence of anti-B19-specific antibodies by ELISA. B19 DNA was detected in plasma and peripheral blood leukocyte (PBL) samples of all patients and controls as well as in synovial fluid cells of 38 RA patients by nested polymerase chain reaction. Disease activity and clinical manifestations were determined in RA patients with and without markers of B19 infection.

Results. IgM anti-B19-specific antibodies were detected in 24.0% of RA patients; B19 DNA was found in plasma and/or PBL, synovial fluid cells in 34.0% (34 patients); in 14.0% of the cases (14 patients) both markers were found. In blood donor controls, anti-B19 IgM antibodies were observed in 16.0% (15 donors) and B19 DNA in 6.4% (6 donors); all donors with detectable B19 genomic DNA were IgM-positive. The disease activity in patients with and without B19 infection was similar, while the frequency of clinical complications was significantly higher in the patients with anti-B19 IgM antibodies. Moreover, liver failure and sicca syndrome were observed in the viremic patients only.

Conclusion. Our study confirms observations regarding a high prevalence of B19 DNA in patients with RA, and a possible role of this viral infection in the pathogenesis of RA. (First Release May 15 2008; J Rheumatol 2008;35:1265–70)

Key Indexing Terms:
HUMAN PARVOVIRUS B19 RHEUMATOID ARTHRITIS PREVALENCE
DISEASE ACTIVITY LIVER FAILURE

Human parvovirus B19 (B19) is a ubiquitous DNA virus, which has been associated with erythema infectiosum in children, hydrops fetalis in pregnant women, aplastic crisis in patients with hemolytic disorders, and persistent pure red cell aplasia in immunocompromised persons[1]. Although replication of B19 is restricted to differentiating and proliferating erythroid precursors in human bone marrow, recent studies showed that the virus can persist in multiple tissues and has been implicated in hepatitis, fulminant liver failure, glomerulonephritis, dermatomyositis, neurological disorders, and myocardial diseases[2-12]. Several clinical studies have linked B19 infection with chronic arthritis, including adult rheumatoid arthritis (RA) and juvenile RA. RA is a chronic systemic autoimmune disease of unknown etiology that afflicts 1.0% of the general population in most countries[13]. The evidence for a role of B19 in the pathogenesis of RA has been demonstrated by high detection frequency of B19 DNA in patients with RA, detection of viral DNA in RA synovial tissues, presence of B19 DNA and protein expression in synovium of RA patients with active synovial lesions, demonstration of infectious virus in macrophages and T and B lymphocytes in patients with RA, development of classical RA after acute B19 infection, the appearance of susceptibility to collagen II (CII)-induced arthritis in B19 transgenic mice, the increased invasiveness of synovial fibroblasts infected by B19, and cross-reactivity of anti-B19 IgG with human CII[14-21]. However, other studies found weaker or no association of B19 with RA[22-26]. We investi-

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gated the prevalence of B19 infection in patients with RA and the relationship between B19 infection and clinical symptoms.

MATERIALS AND METHODS

Patients and specimens. One hundred patients with RA, 72 (72.0%) women and 28 (28.0%) men, were enrolled in this investigation. Their ages varied between 21 and 81 years (mean 55 yrs). Paired blood and synovial fluid samples were collected from 38 patients. All patients were recruited from the Rheumatology Department of Riga Eastern Hospital, Clinic "Linezers," Riga, and fulfilled the American College of Rheumatology criteria for active RA[27]. Sixty-eight (68.0%) RA patients had received a disease modifying antirheumatic drug (DMARD) and/or glucocorticoid therapy. The 32 RA patients without DMARD or glucocorticoid therapy who had received only analgesics and/or nonsteroidal antiinflammatory drugs were defined as the patients without specific therapy.

Ninety-four apparently healthy blood donor controls, 48 (51.0%) men, 46 (48.9%) women, aged 19–58 years (mean 35 yrs) were examined as a control group. The specimens from the RA and control groups were collected at the same time.

The study was approved by the local ethics committee and informed consent was obtained from all patients and controls.

Laboratory monitoring. B19 DNA analysis was carried out on samples from peripheral blood leukocytes (PBL), cell-free plasma, and synovial fluid cells. DNA was purified by proteinase K digestion overnight and extraction by standard phenol-chloroform technique. In the cell-free plasma samples glycogen was used as a carrier. To assure the quality of the PBL DNA and to exclude possible contamination of plasma by cellular DNA, a ß-globin gene polymerase chain reaction (PCR) was carried out. B19 genomic DNA was detected by the nested PCR that amplifies the VP1 region as described[28]. PCR amplification was performed in the presence of 1 µg of PBL DNA and 10 µl of plasma DNA (corresponding to 100 µl plasma). B19-negative DNA were used as a negative control, and DNA from viremic serum (kindly provided by Prof. K. Hedman, Department of Virology, Heartman Institute, University of Helsinki) as a positive control. Water controls were included after every third sample in each experiment.

Plasma samples were tested for B19 IgG and IgM antibodies using the anti-VP2 enzymatic immunoassay (Biotrin, Dublin, Ireland).

Clinical characteristics of RA patients. The RA patients were divided into 5 groups according to the markers of B19 infection. The patients with anti-B19-specific IgM antibodies constituted the first group. Patients with negative results for virus-specific IgM antibodies formed the second (IgG-positive, PCR-positive), third (IgG-positive, PCR-negative), fourth (IgG-negative, PCR-positive), and fifth (IgG-negative, PCR-negative) groups. All groups were compared with respect to age, duration of disease, number of affected joints, presence of erosive arthritis, and erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and rheumatoid factor as well as hemoglobin values and clinical complications.

Statistical analysis. Fisher's exact test (2-tailed) was used in the statistical analysis. A p value < 0.05 was accepted as statistically significant.

RESULTS

Prevalence of B19-specific antibodies. Testing of plasma samples for presence of B19-specific humoral immune reactions showed that 79 out of 100 (79.0%) patients and 73 out of 94 (77.7%) healthy controls had IgG antibodies (Table 1).

IgM antibodies were detectable in plasma of 24 (24.0%) patients (Table 1); 20 of the 24 (83.3%) patients had both anti-B19 IgM and significant levels of anti-B19 IgG antibodies (exceeding the cutoff value by a factor of 3 to 18), while 4 were positive for anti-B19 IgM antibodies only. In the control group 15 out of 94 (16.0%) showed IgM antibodies and 4 of them were IgG-negative.

Detection of B19 DNA sequence. Viral DNA was detected in samples from 34 (34.0%) patients: in plasma in 15; in plasma and PBL, one; in PBL in 13; in PBL and synovial fluid cells in 3; and in synovial fluid cells in 2. The results demonstrated that 16 (16.0%) of the 100 patients had viremia, since the viral DNA could be amplified from cell-free plasma samples (Table 1).

Fourteen out of 16 viremic patients were IgM-positive (12 of them also had IgG antibodies), but 2 did not have anti-B19-specific antibodies. Four of 34 (11.8%) patients with B19 genomic sequence in peripheral blood samples (2 in plasma, 2 in PBL) were negative for both IgM and IgG antibodies, while 16 (with B19 DNA in PBL and/or synovial fluid cells) were negative for IgM antibodies only. Ten patients with anti-B19 IgM antibodies in plasma did not have B19-specific sequences in plasma or in PBL DNA, and 2 of them were IgG-negative.

Thus, among RA patients examined, 14 (14.0%) had both anti-B19-specific IgM antibodies and viral DNA in plasma, 10 (10.0%) had IgM antibodies without detectable B19 DNA in peripheral blood, 16 (16.0%) had anti-B19 IgG antibodies and detectable viral DNA in PBL and/or synovial fluid cells, 43 (43.0%) had IgG class antibodies only, 4 (4.0%) were without anti-B19-specific antibodies but had detectable B19 DNA in plasma or PBL, and 13 (13.0%) had no markers of B19 infection. There was no significant difference in the frequency of B19 infection markers between the RA patients with and those without DMARD and/or glucocorticoid therapy (Table 2). This finding provides evidence that the specific therapy had no significant effect on the positive rate of virus-specific antibodies and B19 DNA positivity.

In the controls, the presence of B19-specific sequence in cell-free plasma and PBL samples was detected in 2 (2.1%) and 4 (4.3%) cases, respectively (Table 1). Viremia (B19 genomic sequence in cell-free plasma) was observed in controls with anti-B19 IgM class antibodies only, while viral genomic DNA was found in PBL of 2 donors with virus-specific IgM and IgG antibodies, and in 2 with anti-B19-specific IgM antibodies only.

There was a significant difference between the RA patients and the control group for B19 DNA positivity (34% vs 6.4%, respectively; $p = 0.0000018$).

Clinical significance of parvovirus B19 infection in RA. The relationship between B19 infection and clinical manifestations was studied in patients with RA. Patients had been divided into the 5 groups according to markers of B19 infection: Group 1 included 24 patients with anti-B19 IgM antibodies; 14 had both IgM antibodies and B19 DNA in plasma (12 of them were also IgG-positive) but 10 had anti-B19 IgM antibodies only (8 of them were also IgG-positive). Group 2 included 16 patients with anti-B19-specific IgG

Table 1. Markers of B19 infection in RA patients and control group.

Groups and Material	Anti-B19 IgM	Anti-B19 IgG	B19 DNA Plasma	B19 DNA PBL	B19 DNA Synovial Fluid Cells
RA patients					
Blood, N = 100 (%)	24 (24.0)	79 (79.0)	16* (16.0)	17** (18.0)	
Synovial fluid cells, N = 38 (%)					5 (13.1)
Controls					
Blood, N = 94 (%)	15 (16.0)	73 (77.7)	2* (2.1)	4** (4.3)	

Frequency of viral DNA in plasma and PBL of RA patients was significantly higher compared with controls: * p = 0.00086, ** p = 0.0049.

Table 2. Prevalence of B19 infection markers in RA patients with and without disease modifying antirheumatic drug and/or glucocorticoid therapy.

Markers of B19 Infection	Patients Received Specific Therapy, n = 68 (%)	No Specific Therapy, n = 32 (%)
IgM	15 (22.1)	9 (28.1)
DNA	19 (27.9)	15 (46.9)
IgG	54 (79.4)	25 (78.1)
No markers	10 (14.7)	3 (9.4)

antibodies and B19 DNA in PBL; Group 3 comprised 43 patients with anti-B19 IgG antibodies only; Group 4: 4 patients without anti-B19-specific antibodies but with detectable B19 DNA in peripheral blood; and Group 5: 13 patients without markers of B19 infection. The clinical characteristics of these RA patients are shown in Table 3. Most of the patients had received DMARD and/or glucocorticoid therapy, which was similar in all groups (those with markers of B19 infection and those without). In Group 1, 15 (62.5%) patients, in Group 2, 8 (50.0%), in Group 3, 32 (74.4%), in Group 4, 3 (75%), and in Group 5, 10 (76.9%) patients had received therapy. Among those who had not had specific therapy there were 29/87 (33.3%) and 3/13 (23.1%) patients with and without B19 infection markers, respectively.

The main criteria for presence of clinical disease activity were ESR and CRP in serum[15]. There were no significant differences between patient groups with respect to age, disease duration, number of affected joints, and presence of erosive arthritis as well as ESR and CRP values. Rheumatoid factor was detected in 8 out of 13 (61.5%) patients without markers of B19 infection (Group 5), in 18 out of 24 (75.0%) patients with the virus-specific IgM antibodies (Group 1), in 12 of 16 (75.0%) patients with anti-B19 IgG antibodies and viral DNA in PBL (Group 2), in 34 of 43 (79.1%) patients with the anti-B19-specific IgG antibodies only (Group 3), and in 3 of 4 patients with no virus-specific antibodies but with the presence of a virus genomic sequence in plasma or PBL DNA (Group 4).

Anemia was observed in all patient groups (with and without markers of B19 infection): in 51.5% (13/24) of patients in Group 1, 56.3% (9/16) of Group 2, 53.5% (23/43) of Group 3, 75.0% (3/4) of Group 4, and 7.7% (1/13) of Group 5. The mean hemoglobin value was 91 ± 20, 95 ± 21, 102 ± 14, 96 ± 10, and 88 ± 28 g/l in Groups 1, 2, 3, 4, and 5, respectively. Although anemia occurred prefer-

Table 3. Clinical details of RA patients with B19 infection.

Clinical Characteristics/Therapy	Group 1 IgM+*, n = 24	Group 2 IgM–, IgG+, PCR+, n = 16	Group 3 IgM–, IgG+, PCR–, n = 43	Group 4 IgM–, IgG–, PCR+, n = 4	Group 5 IgM–, IgG–, PCR–, n = 13
Mean age, yrs	53 ± 29	54 ± 23	55 ± 23	55 ± 6	57 ± 11
Mean disease duration, yrs	9 ± 6	7 ± 6	8 ± 7	8 ± 4	8 ± 7
Mean no. joints involved	13 ± 9	17 ± 9	13 ± 7	9 ± 6	13 ± 6
Erosive arthritis (%)	19 (79.1)	13 (81.3)	35 (81.4)	3 (75.0)	13 (100)
Mean ESR, mm/h	47.9 ± 10.6	40.1 ± 11.8	42.3 ± 6.7	42.0 ± 19.0	40.0 ± 13.0
Mean CRP, mg/l	25.8 ± 6.0	37.0 ± 10.5	24.0 ± 4.2	23.9 ± 13.5	27.7 ± 9.5
Mean RF, IU/ml	193.0 ± 58.8	416.0 ± 138.7	298.9 ± 17.6	95.4 ± 67.1	129.2 ± 30.2
DMARD (%)	7 (29.1)	2 (12.5)	8 (18.6)	0	2 (15.3)
Glucocorticoid (%)	4 (16.7)	2 (12.5)	3 (3.7)	2 (66.6)	2 (15.3)
DMARD and glucocorticoid (%)	4 (16.7)	4 (25.0)	18 (41.8)	1 (33.3)	6 (46.2)
No specific therapy, (%)	9 (39.1)	8 (47.1)	11 (25.6)	1 (25.0)	3 (23.1)

* IgM-positive patients including 14 with both anti-B19 IgM and B19 DNA (12 of them were IgG-positive) and 10 without detectable B19 DNA (8 were IgG-positive).

entially in the patients with markers of B19 infection (Groups 1, 2, 3, and 4), no significant differences in severity of anemia were observed between the patients with and those without infection markers. Moreover, other reasons for anemia, for example, deficiency of iron or vitamin B12, could not be excluded.

Among the patients with markers of B19 infection, complications were observed in 10/24 (41.7%; Group 1) patients with IgM class antibodies (one had polyneuropathy accompanied by myopathy, one kidney and liver failure, 4 polyneuropathy, 2 liver or kidney failure, one myopathy, and one had Sicca syndrome); in 3/16 (18.8%) patients (Group 2) with anti-B19 IgG antibodies and viral genomic sequence in PBL DNA (2 had kidney failure and one myopathy); as well as in 8/43 (16.2%) patients (Group 3) with anti-B19 IgG antibodies only (2 had polyneuropathy accompanied by kidney failure, 2 polyneuropathy and myopathy, one myopathy, and 3 polyneuropathy). Polyneuropathy was also observed in one out of 13 (7.7%) patients without B19 infection (Group 5). No clinical complications were observed in 4 patients without virus-specific antibodies but with the virus genomic sequence in plasma or PBL DNA (Group 4). The rate of complications we observed was significantly higher ($p = 0.01164$) in the patients with anti-B19 IgM antibodies (Group 1) compared to the patients of all the IgM-negative groups [10/24 (41.7%) vs 12/76 (15.8%), respectively]. Liver failure and sicca syndrome were identified in the viremic patients only.

DISCUSSION

Although only the association of acute joint manifestations and B19 infection is well established, the relationship between B19 infection and RA, and the clinical manifestations of B19 infection in patients with RA, remain obscure. Our data showed the association of B19 infection with RA: the frequency of viremia and the rate of detection of B19 DNA in PBL of RA patients significantly exceeded those in healthy controls ($p = 0.00086$ and $p = 0.0049$, respectively).

The positive rates of anti-B19 IgG antibodies were similar between RA patients and controls in our study. This finding is different from previous data showing that the rate of anti-B19 IgG antibodies is age-dependent[29]. This discrepancy could be due to the fact that 4 patients, despite the presence of B19 DNA in peripheral blood (in plasma in 2 and in PBL in 2), did not have anti-B19 antibodies. The lack of anti-B19 antibodies has also been described for patients with systemic lupus erythematosus with B19 DNA in sera[30]. Moreover, we cannot exclude that one out of the 4 IgM-positive, IgG-negative patients, or all of them, could represent, not acute B19 infection, but chronic infection with failure to progress to maturation of the humoral immune response to B19; this has been described for occasional patients[31-33].

It is known that B19 viremia occurs 1 week after exposure and usually lasts about 5 days; B19-specific antibodies are detectable later in the viremic stage (at about Day 10 or 12) and can persist for up to 5 months; and specific IgG antibodies are detectable about 15 days postinfection, remain high for several months, and persist over the long term. During the active phase of infection B19 viremia is identified from the blood with the appearance of IgM and IgG antibody responses[29]. Recent studies revealed that clearance of B19 viremia (detectable B19 DNA in serum) is slower than previously thought; and after acute infection, viremia was maintained for more than 128 weeks after development of anti-B19 IgG antibodies[34]. Moreover, in spite of the development of B19-specific immune reactions, about 20% of all patients with B19 infection show prolonged viral persistence, with detectable viral genomic DNA in bone marrow or other organs (synovial tissues, liver, skin, myocardium) for several years after infection[2-5,26,35,36]. The positive rate of B19 DNA in PBL — 18% — could be explained by the presence of B19 over an extended period in lymphocytes and/or macrophages of RA patients, and is in accord with data from Takahashi, *et al*[16]. Prolonged persistence of B19 and transcription of B19 NS1 mRNA in the monocytic cell line U937 has also been described[16,37]. We assume that the target cells for B19 in PBL are monocytes/macrophages, which can serve as a reservoir for virus migration to different sites, including the synovial compartment, myocardium, liver, kidney, and other organs, where it can persist and promote local inflammation.

Summarizing the clinical data of RA patients with B19 infection, we suggest that B19 may be responsible for liver dysfunction, or could be a trigger for development of this disorder upon therapy. Arguments for involvement of B19 in liver injury have been reported[4,7,38,39]. Moreover, it has been shown that B19 infection induces apoptosis in primary hepatocytes and that production of the B19 nonstructural protein NS1 is sufficient to induce apoptosis in hepatic cells[40,41]. However, taking into account that disease duration in both the patients with liver failure was 12 years, and that both had received the antimetabolite drug methotrexate, the hepatotoxicity of which has been described in occasional RA patients, we cannot exclude that liver failure is a side effect of such therapy, or a complication of the disease itself[42]. Nevertheless, liver failure was not observed in the patients without active infection, although 35/76 (46.6%) patients had received the same antimetabolite drug, and the disease duration in 17/35 (48.6%) of them was 12 years or more. Other disorders observed among RA patients also occurred more commonly in patients with active B19 infection. Although no significant correlation between disease activity and active B19 infection was found, we cannot exclude that B19 may be directly (via cytotoxicity) or indirectly (via immunologically mediated or inflammatory injury) involved in tissue damage.

The finding that 20 out of 24 (83.3%) patients with anti-B19 IgM antibodies also had significant levels of anti-B19

IgG antibodies suggests that most of these patients developed adequate humoral immune response to the B19 infection. Two explanations appear plausible for the lack of anti-B19 IgG antibodies in 4 patients: acute B19 infection or failure to progress in maturation of the humoral immune response to B19.

That detectable B19 DNA was present in plasma in 2 patients with RA without virus-specific antibodies suggests that these patients probably were in a pre-seroconversion period of the infection, or they may have had a chronic B19 infection. It has been reported that the lack of neutralizing antibodies has been associated with chronic B19 infection[31]. The 2 other patients with detectable B19 viral sequences in PBL DNA, but with no detectable antiviral antibodies in plasma, would not be able to generate B19-specific IgG. It is reasonable that elimination of the virus by the immune system may be restricted to the immunosuppressive effect of the glucocorticoid drug used.

Thus, it is possible that B19 infection may affect the course of RA, leading to multiorgan involvement. However, our findings require confirmation in order to elucidate the clinical significance of B19 infection in RA.

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