Parvovirus B19 infection, hepatitis C virus infection, and mixed cryoglobulinaemia

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Abstract

Background—Infection with human parvovirus B19 (B19) has been reported in a few patients with various vasculitis syndromes. Mixed cryoglobulinaemia (MC), a model of small vessel size vasculitis, may result from numerous infectious diseases, particularly hepatitis C virus (HCV) infection.

Aim—To assess the prevalence of seric B19 infection markers in a large series of patients with MC, with or without HCV infection.

Patients and methods—Sixty four patients were studied: essential MC (EMC, n = 19), MC associated with non-infectious diseases (non-essential MC, n = 9), and patients with HCV infection with (HCV-MC, n = 18) or without MC (HCV-no-MC, n = 18). Patients were considered to have MC if two successive determinations of their serum cryoglobulin concentration were above 0.05 g/l. Serum samples were analysed for specific IgG and IgM antibodies to B19 by enzyme immunoassay. B19 DNA detection was performed by polymerase chain reaction using a set of primers located in the VP1 gene, separately in serum and in cryoprecipitates to investigate a possible capture of B19 DNA in cryoprecipitate. The study also looked for a possible enrichment for of IgG antibodies to B19 in MC.

Results—The presence of specific IgG antibodies to B19 was found in 68% EMC, 56% non-essential MC, 78% HCV-MC, and 78% HCV-no-MC. No patient of either group had specific IgM antibodies to B19, or B19 DNA in serum or in cryoprecipitate. Overall, IgG antibodies to B19 were found in 46 of 64 (72%) serum samples, a prevalence quite similar to the prevalence in general adult population (> 60 %). A specific enrichment of IgG antibodies to B19 in the MC was not found.

Conclusion—These results suggest that B19 infection is neither an aetiological factor of EMC, nor a cofactor that may lead to MC production in patients with chronic HCV infection.

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Case reports of B19 infection associated with neuropathies, vasculitis or erosive polyarthritides suggest that B19 could be an aetiological factor of some vasculitis syndromes.^{3 4}

The syndrome of mixed cryoglobulinaemia (MC) is characterised by the clinical triad of purpura, arthralgia, and asthenia associated with cryoglobulins composed of different immunoglobulins.⁵ Widespread vasculitis usually involves small and medium size vessels, particularly in the nervous system and in the skin.⁶ MC has been associated with numerous diseases—autoimmune, non-viral infectious or malignant haematological disorders—but may also occur in their absence and is then called “essential” MC. It has been demonstrated that 60–90% of patients with previously “essential” MC have serological evidence of hepatitis C virus (HCV) infection, suggesting a causative role of HCV.^{7–10}

To investigate the prevalence of serological B19 infection markers in patients with MC, we looked for specific IgG and IgM antibodies to B19, and B19 DNA in serum and cryoprecipitate in a large series of patients with MC, with or without HCV infection.

Methods

PATIENTS

We studied 64 French white patients. Nineteen patients aged 52 (3) years (mean (SD)) had a symptomatic MC without underlying disease such as autoimmune, infectious or malignant haematological disorders (group 1 = essential MC). In group 2, nine patients aged 51 (2) years had a MC associated with a known cause of production (non-essential MC): systemic lupus erythematosus (n = 3), malignant hemopathy (n = 3), infective endocarditis (n = 2), and mediterranean fever (n = 1). Groups 3 and 4 included patients with chronic HCV infection. Chronic HCV infection was defined by the presence of alanine aminotransferase activities more than twice the upper limit of normal range, anti-HCV antibodies detected by third generation test (ELISA, RIBA), liver biopsy findings compatible with chronic hepatitis C, and no other cause of liver dysfunction (for example, chronic hepatitis B, autoimmune hepatitis, primary biliary cirrhosis, etc). In group 3, 18 patients aged 50 (3) years had a chronic HCV infection and a symptomatic MC. Patients were considered to have a significant cryoglobulin if they had a minimum serum cryoglobulin concentration of 0.05 g/l at two determinations (see methods). In group 4, 18 patients aged 52 (2) years had a chronic HCV infection without MC.

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Infection with human parvovirus B19 (B19) has a diverse range of clinical manifestations including erythema infectiosum, polyarthropathy, transient aplastic crisis, hydrops fetalis and fetal death, and chronic infection and anaemia in immunocompromised hosts.^{1 2}

Table 1 Distribution of parvovirus B19 infection serological markers in patients with essential MC (group 1), MC with a known cause (group 2), and in patients with chronic hepatitis C virus infection with (group 3) or without MC (group 4)

	Group 1	Group 2	Group 3	Group 4
Patient number	19	9	18	18
Underlying disease	no	miscellaneous*	HCV	HCV
MC status	+	+	+	—
B19 IgG in serum	13	5	14	14
B19 IgM in serum	0	0	0	0
B19 DNA in cryoprecipitates	0	0	0	—
B19 DNA in serum	0	0	0	0

HCV = hepatitis C virus, MC = mixed cryoglobulinaemia, B19 = parvovirus B19 infection, *miscellaneous = autoimmune, infectious or malignant haematological disorders.

STUDY DESIGN

Cryoglobulins were isolated from serum samples, purified, and characterised by immunoblotting at 37°C as previously described.¹¹ After immunochemical analysis, cryoglobulins were classified according to Brouet *et al.*¹²: type I cryoglobulins are single monoclonal immunoglobulins; types II and III are mixed cryoglobulins, composed of different immunoglobulins, with a monoclonal component in type II and only polyclonal immunoglobulins in type III. In this study, all patients with cryoglobulinaemia had type II or type III MC. Anti-HCV antibodies were detected in all patients by third generation tests (ELISA, Ortho and RIBA Chiron, Diagnostic Systems).

After collection at 37°C, serum samples were kept frozen at -80°C until analysis. Serum samples were then thawed and analysed at 37°C for specific IgG and IgM antibodies to B19 by enzyme immunoassay (Biotrin International, Lyon, France). B19 DNA detection in serum and cryoprecipitates was performed by polymerase chain reaction using a set of primers located in the VP1 gene (01–376: 5'-GTG CTT ACC TGT CTG GAT TGC 3' and 02–377: 5'-GCT AAC TTG CCC AGG CTT GT 3'). The 402 bp polymerase chain reaction products were analysed on a 1.5% agarose gel. After southern blotting, polymerase chain reaction products were hybridised with a DIG-dUTP labelled oligonucleotide probe (Probe 2560–2600: 5'-AAT ATT AAA AGA TCA TTA TAA TAT TTC TTT AGA TAA TCC CC 3'). Positive (at a 10⁻⁶ dilution) and negative controls were systematically included in the polymerase chain reaction.

In patients with MC, we also looked for B19 DNA separately in cryoprecipitate and in serum supernatants, to investigate a possible capture of B19 DNA in the cryoprecipitate alone. In six selected MC patients of different groups—according to the presence of a sufficiently high cryoglobulin concentration (above 0.8 g/l)—we also looked for a possible enrichment of IgG antibodies to B19 in the cryoglobulins. Briefly, we quantified the concentration of IgG in the serum and the cryoprecipitates by nephelometry (BN II Behring, Marburg, Germany). Then, we used a quantitative assay for IgG antibodies to B19. Finally, we calculated the ratio: IgG antibodies to B19 in cryoprecipitate/IgG antibodies to B19 in serum.

Statistical analysis used χ^2 or Fisher's exact test for comparisons of percentage. Probability

values of less than 0.05 were considered statistically significant.

Results

Table 1 summarises the distribution of B19 infection markers in the study patients. The presence of specific IgG antibodies to B19 was found in 13 of 19 (68%) essential MC, 5 of 9 (56%) non-essential MC, 14 of 18 (78%) HCV-MC, and 14 of 18 (78%) HCV without MC. However, no patient of either group had specific IgM antibodies to B19, or B19 DNA in serum or in cryoprecipitate. In HCV patients (groups 3 and 4), the prevalence of B19 infection markers was not different whether they have or not MC. In the six selected patients with MC, the ratio IgG antibodies to B19 in cryoprecipitate/IgG antibodies to B19 in serum was from 0.34 to 1.12.

Discussion

We found a high prevalence (56 to 78%) of IgG antibodies to B19 in patients with MC. However, whatever the aetiology of MC, no patient had IgM antibodies to B19. B19 DNA was not detected in serum or in cryoprecipitates of patients, whatever the group. Also, no MC patient had a serological marker of active B19 infection.

The high prevalence of IgG antibodies to B19 (46 of 64=72%) found in serum is not surprising because it is quite similar to the prevalence in general adult population (>60% after 50 years).^{13,14} The prevalence of IgG antibodies to B19 was identical in HCV patients with or without MC. We studied the possibility of false negative results for active B19 infection markers in patients with MC because of B19 DNA capture in cryoprecipitate. We ruled out this hypothesis because when we looked for the presence of B19 DNA separately in serum and in cryoprecipitate, the results remain negative in all cases. We did not find a specific enrichment of IgG antibodies to B19 in cryoprecipitate, suggesting that parvovirus B19 had no role in the MC production.

MC has been associated with numerous diseases—autoimmune, infectious or malignant haematological disorders—but may also occur in their absence and is then called “essential” MC. During acute infections, MC is generally transient and it is rarely symptomatic. MC has been reported with a high frequency in chronic infectious diseases such as subacute bacterial endocarditis,¹⁵ lepromatous leprosy,¹⁶ kala-azar,¹⁷ infectious mononucleosis,¹⁸ Lyme arthritis,¹⁹ HIV, cytomegalovirus,²⁰ and Epstein-Barr virus infection.²¹ All the common hepatitis viruses (A, B, and C) may also lead to the production of MC, but HCV is by far the most frequent infectious aetiological factor.^{22–24} We and others have demonstrated that more than 70% of patients with previously “essential” MC have serological evidence of HCV infection, and many data suggest a causative role of HCV.^{7–10} Conversely, MC is particularly frequent in HCV chronic infection where it is found in up to 60% of patients.^{23,24} However, the search for factors that explain the MC production in these HCV patients is still going on. We previously reported

that HCV patients with MC compared with those without MC had a longer duration of HCV infection and more often liver cirrhosis.¹⁰⁻²⁵ The possibility of a HBV coinfection has been ruled out. This study cannot support the hypothesis for a role of a B19 active infection in the mechanisms of MC production either as a single factor in essential MC or as a cofactor in HCV-MC. A possible link between B19 infection and some vasculitis syndromes have been reported in only a few case reports.⁴⁻²⁶⁻²⁹ However, all the studies searching systematically for markers of B19 active infection in patients with polyarteritis nodosa³⁰ or Kawasaki syndrome³¹⁻³² were negative. Thus, our negative results on B19 infection in MC, a model of small and medium size artery vasculitis, are not surprising.

In conclusion, these results suggest that B19 infection is neither an aetiological factor of essential MC, nor a cofactor that may lead to MC production in patients chronically infected by HCV.

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