Lack of association between B19 or V9 erythrovirus infection and ANCA-positive vasculitides: a case–control study

A. Eden, A. Mahr*, A. Servant1*, N. Radjef, S. Amard1, L. Mouthon, A. Garbarg-Chenon1 and L. Guillevin

Objectives. To examine the potential association of human B19 or V9 erythrovirus infection and onset of ANCA-positive vasculitides.

Methods. We tested the sera of 13 adults with newly diagnosed ANCA-positive vasculitides. Each was age- and sex-matched to three sera obtained from healthy controls. All samples were tested for B19- and V9-specific immunoglobulin (Ig) G and IgM antibodies (Ab) (third-generation ELISA), and B19 or V9 DNA was sought with the polymerase chain reaction. Statistical analysis was performed by conditional logistic regression.

Results. Patient diagnoses comprised six cases of Wegener’s granulomatosis, six of microscopic polyangiitis and one of Churg–Strauss syndrome. IgG Ab to B19 were detected equally in patient and control sera (77 and 79% respectively) (odds ratio = 0.84, P = 0.84). All 13 cases and 39 controls were negative for IgM Ab and viral DNA.

Conclusion. These results suggest that neither acute nor chronic B19 or V9 infection is an aetiological factor in ANCA-associated vasculitides.

KEY WORDS: Erythrovirus, B19, V9, ANCA, Vasculitis.
Patients and methods

We tested sera from an empirically determined number of 13 patients with ANCA-positive WG, MPA or CSS diagnosed according to the 1990 criteria of the American College of Rheumatology (ACR) [19, 20] or the Chapel Hill Consensus Conference (CHCC) nomenclature for vasculitides [21]. These sera had been drawn at the time of diagnosis and before onset of immunosuppressive therapy, and had been stored at -80°C until being assayed. Every case was matched for age (± 3 y) and sex to three healthy controls. Control sera were obtained either from blood donors after informed consent had been obtained or from healthy hospital staff physicians.

Sera were screened for specific immunoglobulin (Ig) G and IgM antibodies (Ab) to B19 and for erythrovirus DNA. A highly sensitive commercial enzyme-linked immunosorbent assay (ELISA) (Parvovirus B19 IgG or IgM Enzyme Immunoassay, third generation; Biotrin, Dublin, Ireland) [22–24] was used to detect B19-specific Ab. This test uses purified parvovirus protein 2 (VP2) produced in the baculovirus system as the source of antigen cross-reacting with Ab directed against V9 [25]. The polymerase chain reaction (PCR) was used to search for B19 and V9 DNA. DNA was extracted from serum using the QIAamp DNA Mini Kit (Qiagen, Courtaboeuf, France). Screening for the presence of B19 and V9 DNA was performed with an erythrovirus consensus PCR assay using primers e1905f (5′-TGCAGATGCCCTCCACCC) and e1987r (5′-GCTGCTTTCACTGAGTTCTTC) located in the NS1 gene of B19 or V9 viruses and 1.5 U of AmpliTaqGold™ (Applied Biosystems, Villebon, France). Hot-start amplification was performed in a Gene Amp PCR System 9700 (Applied Biosystems) under the following conditions: one cycle at 94°C for 6 min, five cycles at 94°C for 30 s, 55°C for 1 min, and 72°C for 1 min, 45 cycles at 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s followed by a final elongation step at 72°C for 7 min. The PCR products (103 base pairs) were analysed by electrophoresis in 2% agarose gels and were hybridized with a B19/V9-specific biotinylated DNA probe e1954fp (5′-ACCCATATCGACGACAGTTGGTGTTGAAAGCCTGAA) using a commercial assay (DNA Enzyme Immunoassay Kit; Diasorin, Antony, France). The NS1-PCR assay has a detection threshold of 1 viral DNA copy per PCR reaction with 100% sensitivity [10]. Positive and negative controls were included in every PCR run; they consisted of a serum sample containing a B19 isolate and water respectively. All commercial assays were used according to the manufacturer’s recommendations.

Cases and controls were compared with respect to the presence of IgG and/or IgM Ab and DNA detection by PCR. Statistical analysis was performed by conditional logistic regression (1:3 matched study) using the PROC PHREG procedure of the SAS Statistical Package, version 8.12 (SAS Institute Inc., Cary, NC, USA).

Results

The 13 patients (mean age 50.1 ± 11.1 yr, male:female sex ratio: 1:6) comprised eight with WG, four with MPA and one with CSS. All the WG and CSS patients fulfilled the ACR criteria [19, 20] and the MPA patients met the CHCC nomenclature [21]. Histological proof was obtained for 11 patients with evidence of necrotizing vasculitis (n = 4) and/or pauci-immune glomerulonephritis (n = 5); the remaining two patients (patients 6 and 7) were diagnosed with WG on the basis of their clinical manifestations. Indirect immunofluorescence assay of ANCA identified eight patients with cytoplasmic labeling [including six who were proteinase 3 (PR3)- and one who was myeloperoxidase (MPO)-specific, by ELISA] and five with perinuclear labeling (four of whom recognized MPO). The demographic, clinical and immunological data of these 13 cases are summarized in Table 1.

IgG Ab to B19 were detected in 79% of all subjects, with comparable percentages for cases (77%) and controls (79%) (odds ratio = 0.84, 95% confidence interval = 0.17–4.22, P = 0.84). None of these sera were positive for IgM Ab to B19. B19 and V9 DNA were never detected in any serum sample.

Discussion

The results of our study do not support the idea that B19 or V9 infection is an aetiological factor in ANCA-positive vasculitides. The fact that the frequencies of IgG Ab in our patients and controls were similar suggests that both groups had been similarly exposed to these erythroviruses. Moreover, none of the patients had IgM Ab or detectable viraemia, which indicates the absence of recent or chronic erythrovirus infections.

The role of B19 in various vasculitides remains a matter of controversy. Evidence for such an association has come from epidemiological studies suggesting a chronological link between B19 outbreaks and the incidence of giant-cell arteritis [26]. Authors of a case–control study reported a significantly higher B19 DNA PCR detection rate in the temporal artery walls of patients with giant-cell arteritis [27], although those findings were not confirmed by another study [28]. A serological study detected significantly higher B19 IgM Ab rates in Behçet’s disease patients than in controls [29]. Conversely, an uncontrolled study conducted on 42 WG patients with unspecified ANCA status did not obtain serological or molecular evidence of recent B19 infection [30], which would be in agreement with our negative findings.

The pathogenic mechanism that has been advanced to explain induction by B19 of the vasculitic process is based on the deposition of immune complexes or, more specifically, on direct viral invasion of the vessel wall through the presence of the B19 receptor, P antigen, on the surface of endothelial cells [11]. The isolation of ANCA from four patients with ongoing B19 infections [18] consequently suggests an additional mechanism by which B19 could trigger the onset of vasculitis. However, the pathogenicity of ANCA detected in infectious circumstances, even for ANCA whose specificity can be determined by antigen-specific ELISA [31], is probably limited. In this respect, it is pertinent to note that acute B19 infection has also been associated with the occurrence of other autoantibodies, such as antinuclear and anticardiolipin antibodies, while no evidence was found for a role of B19 in systemic lupus erythematosus [24].
<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Clinical manifestations</th>
<th>Histological proof</th>
<th>ANCA labelling pattern</th>
<th>ELISA (titre)</th>
<th>B19 Ab</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MPA</td>
<td>30</td>
<td>F</td>
<td>Glomerulopathy</td>
<td>Glomerulonephritis (segmental and focal)</td>
<td>P</td>
<td>MPO (&gt; 200 U/ml)</td>
<td>Neg.</td>
<td>Neg.</td>
</tr>
<tr>
<td>2</td>
<td>WG</td>
<td>37</td>
<td>M</td>
<td>Recurrent sinusitis, alveolar haemorrhage, renal insufficiency, arthritis</td>
<td>Glomerulonephritis (extracapillary)</td>
<td>C</td>
<td>PR3 (95 U/ml)</td>
<td>IgG</td>
<td>Neg.</td>
</tr>
<tr>
<td>3</td>
<td>WG</td>
<td>40</td>
<td>F</td>
<td>Nasal discharge, haemorrhage, glomerulopathy, arthralgia</td>
<td>Glomerulonephritis (extracapillary, necrotizing), periglomerular granuloma</td>
<td>P</td>
<td>MPO (177 U/ml)</td>
<td>Neg.</td>
<td>Neg.</td>
</tr>
<tr>
<td>4</td>
<td>CSS</td>
<td>43</td>
<td>M</td>
<td>Asthma, pulmonary infiltrate, eosinophilia, pericarditis, renal insufficiency, neuropathy</td>
<td>Glomerulonephritis (extracapillary), necrotizing vasculitis (muscle)</td>
<td>C</td>
<td>MPO (&gt; 200 U/ml)</td>
<td>IgG</td>
<td>Neg.</td>
</tr>
<tr>
<td>6</td>
<td>WG</td>
<td>49</td>
<td>M</td>
<td>Nasal bleeding, pulmonary nodule with cavitation, arthralgia, neuropathy</td>
<td>NA</td>
<td>C</td>
<td>NA</td>
<td>IgG</td>
<td>Neg.</td>
</tr>
<tr>
<td>7</td>
<td>WG</td>
<td>51</td>
<td>M</td>
<td>Recurrent sinusitis, alveolar haemorrhage, renal insufficiency, neuropathy, purpura</td>
<td>NA</td>
<td>C</td>
<td>PR3 (153 U/ml)</td>
<td>IgG</td>
<td>Neg.</td>
</tr>
<tr>
<td>8</td>
<td>MPA</td>
<td>53</td>
<td>M</td>
<td>Alveolar haemorrhage, glomerulopathy</td>
<td>Glomerulonephritis (segmental and focal)</td>
<td>P</td>
<td>NA</td>
<td>IgG</td>
<td>Neg.</td>
</tr>
<tr>
<td>9</td>
<td>WG</td>
<td>56</td>
<td>F</td>
<td>Nasal bleeding, sinusitis, pneumonia-like condensation, neuropathy, retinal ischaemia, peripheral gangrene</td>
<td>Vasculitis, granuloma (lung)</td>
<td>C</td>
<td>PR3 (179 U/ml)</td>
<td>IgG</td>
<td>Neg.</td>
</tr>
<tr>
<td>10</td>
<td>MPA</td>
<td>60</td>
<td>M</td>
<td>Myalgia, pleural effusion, pericarditis</td>
<td>Necrotizing vasculitis (muscle)</td>
<td>P</td>
<td>MPO (35 U/ml)</td>
<td>IgG</td>
<td>Neg.</td>
</tr>
<tr>
<td>11</td>
<td>WG</td>
<td>63</td>
<td>M</td>
<td>Pneumonia-like condensation, pericarditis, glomerulopathy</td>
<td>Glomerulonephritis (extracapillary)</td>
<td>C</td>
<td>PR3 (179 U/ml)</td>
<td>IgG</td>
<td>Neg.</td>
</tr>
<tr>
<td>12</td>
<td>WG</td>
<td>64</td>
<td>M</td>
<td>Nasal bleeding, neuropathy, myalgia</td>
<td>Vasculitis, granuloma (temporal artery)</td>
<td>C</td>
<td>PR3 (&gt; 300 U/ml)</td>
<td>IgG</td>
<td>Neg.</td>
</tr>
<tr>
<td>13</td>
<td>WG</td>
<td>64</td>
<td>F</td>
<td>Nasal discharge, septal perforation, renal insufficiency</td>
<td>Glomerulonephritis (extracapillary)</td>
<td>C</td>
<td>PR3 (80 U/ml)</td>
<td>IgG</td>
<td>Neg.</td>
</tr>
</tbody>
</table>

NA, not available; C, cytoplasmic; P, perinuclear; Neg., negative.
The present study has potential limitations. The small sample size and the exclusively adult population we investigated did not allow us to exclude the possibility that B19 was implicated in individual cases of ANCA-associated vasculitides, particularly in children or young adults [30]. As the natural history of ANCA-associated vasculitides remains unknown, it can be further hypothesized that the interval between the acute triggering infection and vasculitis onset may be long, with signs of recent erythrovirus infection having already subsided in most patients at the time of diagnosis. However, according to this hypothesis we should have observed a higher rate of IgG Ab in patients than in controls. Finally, because we analysed blood and not tissue samples, the possibility that the microorganism we investigated persists chronically in the endothelium and triggers ANCA-positive vasculitides by a local action cannot be excluded. However, according to this scenario we would have expected the injured endothelial cells to release, at least in small amounts, viral particles or DNA into the bloodstream.

We conclude that B19 and the novel erythrovirus V9 do not appear to be a dominant or unique aetiological factor in ANCA-associated vasculitides. Nonetheless, until the causative agent(s) of ANCA-associated vasculitides is discovered, patients with suspected or newly diagnosed ANCA-positive vasculitis should be screened for B19 or V9 infection, either as a putative occasional risk factor or as a differential diagnosis of ANCA-associated disease.

Acknowledgements

The authors are indebted to the following physicians who contributed to the study: M. Debien, A. Groc, A. Krivitzky, O. Lortholary, D. Roulot, J. Salama (Bobigny), Ó. Fain (Bondy), R. Fior (Clamart), X. Belenfant (Montreuil), H. Ben Hadj Amor, J. Moh-Klaren and L.H. Noël (Paris). We are also grateful to Ms J. Jacobson for editorial assistance.

References


