Human parvovirus B19, varicella zoster virus, and human herpes virus 6 in temporal artery biopsy specimens of patients with giant cell arteritis: analysis with quantitative real time polymerase chain reaction


Objective: To evaluate the role of parvovirus B19 (B19), varicella zoster virus (VZV), and human herpes virus 6 (HHV-6) in the aetiopathology of giant cell arteritis (GCA).

Methods: Temporal artery biopsy specimens from 57 patients with GCA and 56 controls were investigated. DNA was obtained by biopsy, and quantitative real time polymerase chain reaction assay performed to establish the prevalence and viral load of B19, VZV, and HHV-6. Amplification of the human β-globin gene was used as internal positive control.

Results: (a) B19 was detected in 31/57 (54%) patients (median viral load 45.2 (25th–75th centiles 0–180.2) copies/µg DNA) vs 21/56 (38%) controls (median viral load 0 (0–66.7) copies/µg of DNA; p = 0.07 for DNA prevalence, p = 0.007 for viral load. Among 31 B19 positive samples, 21 (68%) patients with biopsy proven GCA had >10² B19 copies/µg of DNA vs 5/21 (24%) controls; p = 0.001. (b) No significant difference was found for VZV (p = 0.94 for DNA prevalence; p = 0.76 for viral load) and HHV-6 (p = 0.89 for DNA prevalence; p = 0.64 for viral load) in the GCA group compared with controls.

Conclusion: B19 may have a role in the aetiopathology of GCA, particularly in those patients with high viral load; no evidence was found for VZV and HHV-6.

Recent studies have suggested that persistent infections may be involved in the pathogenesis of giant cell arteritis (GCA), although evidence has been inconclusive. GCA is a disease of unknown aetiology characterised by acute inflammation affecting medium and large arteries in elderly people, with a predilection for branches of the temporal arteries. Multiple environmental and genetic factors probably play a part in susceptibility to GCA. Although certain HLA-DR alleles are correlated with GCA, genetic factors are insufficient to explain the marked variation in geographic prevalence and the temporal fluctuation in the incidence of the disease. Different findings suggest that infectious agents may be involved in the pathogenesis of the disease: parvovirus B19 (B19), human herpes virus 6 (HHV-6), and varicella zoster virus (VZV).

This study aimed at determining B19, VZV, and HHV-6 DNA prevalence; p = 0.64 for viral load) in the GCA group compared with controls.

Patients and methods

We examined TAB specimens from 113 patients suspected of having GCA. Patients, before biopsy, had taken either no drugs for GCA or corticosteroids for less than 2 weeks. Fifty-seven patients (38 women) had biopsy proven GCA (GCA group) and 56 patients (40 women), had no histological evidence of vasculitis (control group). The mean (SD) age of the GCA group was 78 (8.7) years and of the controls 77.6 (7.9) years; p = 0.75. Patients were recruited from the rheumatology service of Hospital Clínico San Carlos (HCSC), Madrid. TAB specimens were collected at the pathology service of HCSC. The investigators who carried out the DNA analysis did not know the clinical and histological diagnoses. The study was approved by the local ethics committee.

DNA extraction

Ten sections 4–6 µm thick taken from each biopsy specimen were deparaffinised by incubating for 5 minutes each in two changes of xylene and for 5 minutes in absolute ethanol. The specimens were dried and rehydrated in distilled water. The DNA was then extracted using Qiagen columns (QIAamp DNA Mini Kit; Qiagen Inc), according to the protocols supplied by the manufacturer; then was quantified by spectrophotometry. In all samples, DNA extraction was successful, as confirmed by the presence of sufficient and non-degraded human DNA.

Quantitative real time PCR

The DNAs were analysed by quantitative real time polymerase chain reaction (q-rtPCR) for the presence of B19, VZV, and HHV-6 genomes and the human β-globin gene as internal control (to ensure that DNAs were suitable for DNA amplification). Primers, TaqMan probes, and reaction conditions for the q-rtPCR assay for VZV, HHV-6, and β-globin have been described previously. A commercial kit (Artus B19 RG RealArt PCR kit) was used for the B19 assay. For each virus three reactions were carried out. The quantitative assessment was performed in a Rotor-Gene 2000 real time cycler (Corbett Research, Sydney, Australia). Cycling conditions were preincubation at 95°C for 15 minutes to activate AmpliTaq gold DNA polymerase, then two step thermocycling (denaturation and anneal/extension) was performed for 40 cycles. The q-rtPCR for B19 was carried out according to the manufacturer’s protocol. With these

Abbreviations: B19, parvovirus B19; GCA, giant cell arteritis; HHV-6, human herpes virus 6; q-rtPCR, quantitative real time polymerase chain reaction; TAB, temporal artery biopsy; VZV, varicella zoster virus.
In the control group (0 genomes/mg of DNA), among the patients with GCA we found two TAB specimens with no histological evidence of vasculitis, only five (24%) patients with biopsy proven GCA had high levels of viral load (>10^2 copies/mg of DNA). One case (5%) had 10^2–10^3 B19 copies/μg of DNA, and 10 (32%) had 10^3–10^4 copies/μg of DNA. Among 21 patients without histological evidence of vasculitis, only five (24%) had 10^2–10^3 B19 copies/μg of DNA. The difference in the number of specimens with more than 10^3 copies between both groups is significant (p = 0.001). Nevertheless, our findings did not confirm any association between histological evidence of GCA and the presence of virus DNA in TAB tissue. Interestingly, we found that about 30% of TAB specimens, with the exception of 54% in the case of B19 in the GCA group, were positive for some viral DNA. This high prevalence of viral DNA might be related to the advanced age of patients and/or to the particular characteristics of arterial tissue but, in most cases, viruses seem to be only innocent bystanders.

In any event, B19 DNA seems to be commonly present in TAB specimens of elderly people with GCA, where it probably persists for years or decades after primary infection; this hypothesis is further supported by the finding that the cellular receptor for B19 (erythrocyte P antigen) is also expressed on endothelial cells. However, although the virus is probably latent in the control group, among patients with biopsy proven GCA a reactivation of B19 latent infection may be a possible disease trigger, particularly in those patients with high viral load.

More effort is needed to understand the mechanisms of the viral reactivation and their relationships with the inflammatory process of the disease: does B19 reactivation lead to
inflammation or inflammation leads to B19 reactivation? Further studies are required to evaluate the exact role of B19 in the aetiology of GCA.

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REFERENCES

Table 3 B19, VZV and HHV-6 viral load ranges in positive patients with GCA and controls

<table>
<thead>
<tr>
<th>Viral load range*</th>
<th>B19 GCA (n = 31)</th>
<th>B19 CG (n = 21)</th>
<th>HHV-6 GCA (n = 20)</th>
<th>HHV-6 CG (n = 19)</th>
<th>VZV GCA (n = 18)</th>
<th>VZV GC (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10^1–10^2</td>
<td>10 (32)</td>
<td>16 (76)</td>
<td>15 (75)</td>
<td>16 (84)</td>
<td>14 (78)</td>
<td>18 (100)</td>
</tr>
<tr>
<td>10^2–10^3</td>
<td>19 (61)</td>
<td>5 (24)</td>
<td>5 (25)</td>
<td>3 (16)</td>
<td>4 (22)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>&gt;10^3</td>
<td>2 (6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
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</tbody>
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*Viral loads are expressed in genomes/μg of DNA, No (%).
CG, control group.