GB VIRUS C IN SYSTEMIC MEDIUM- AND SMALL-VESSEL NECROTIZING VASCULITIDES

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SUMMARY

Background. Vasculitides are diseases of unknown origin in the majority of cases, but sometimes are the consequence of viral infections; for instance, hepatitis B virus (HBV)-related polyarteritis nodosa (PAN) or hepatitis C virus (HCV)-associated cryoglobulinaemia.

Objective. To investigate the role of hepatitis G or GB virus C (GBV-C) in various forms of medium- and small-vessel vasculitides.

Design. Retrospective analyses of sera.

Setting. Tertiary care hospital in Bobigny, France.

Patients. Fifty-six vasculitides: 19 HBV-PAN, 10 PAN without HBV infection, 11 microscopic polyangiitis (MPA), seven Churg–Strauss syndrome (CSS) and nine Wegener’s granulomatosis (WG). Every sample was collected before treatment.

Measurements. GBV-C RNA was detected using a reverse transcription-polymerase chain reaction assay with primers derived from the conserved GBV-C helicase and NS5a regions.

Results. GBV-C was detected in five of the 56 samples (8.9%): four patients with HBV-related PAN and one with MPA; three of these patients (two with HBV-PAN, one with MPA) had been transfused and two HBV-PAN were i.v. drug addicts. GBV-C was not found in CSS or in WG.

Conclusion. GBV-C infection was observed only in patients who had been transfused or who were addicts. This virus is unlikely to have a primary role in vasculitides.

Key words: GB virus C, Hepatitis G virus, Vasculitides, Transfusion, Intravenous drug addicts.
The GBV-C genome was detected by using two reverse transcription-polymerase chain reaction (RT-PCR) methods. PCR was performed using NS5a and NS3 region amplifications. Nucleic acids were extracted from 100 µl of serum by guanidium isothiocyanate and precipitated with isopropanol. The RNA pellet was washed with 70% ethanol and suspended in water containing RNase inhibitor (RNAsin®, Promega). cDNA was generated from viral RNA by reverse transcription using Superscript II reverse transcriptase (Gibco BRL) and a specific primer (7253) of the NS5a region or random hexamers (Perkin-Elmer Cetus). For NS5a amplification, the cDNA was amplified with Gold Taq polymerase (Perkin-Elmer) and two NS5a primers (sense 6884: 5'-CCGAGCCCCTCTTTGTGGTAGTAGC; and antisense 7253: 5'-GCGAATTCTCACAATAGGCTGTATGG). The enzyme activation was conducted for 9 min at 94°C and five cycles of amplification (94°C for 1 min, 50°C for 1 min, 72°C for 1 min) were followed by 35 cycles using an annealing temperature of 60°C. For NS3 amplification, cDNA priming was conducted using a random hexamer, and amplification was performed using touch-down PCR as previously described [8]. Procedures to avoid contamination of samples during nucleic acid extraction and genomic amplification steps were strictly followed. Positive and negative controls were included in every step.

PCR products were analysed by electrophoresis in a 1.2% agarose gel with ethidium bromide staining. The detection of a 370 bp band was considered a positive result.

Southern blot analysis. Gel DNA was transferred onto a nylon membrane (Genescreen plus®). The probe was a 3'-digoxigenin-labelled oligonucleotide for the NS5a region (7083–7107). Hybridization was conducted overnight at 42°C. Washing and revealing were performed as described by the manufacturer (Boehringer Mannheim). Film was exposed for 5–60 min.

GBV-C replication analysis. GBV-C-NS3 and -NS5a gene amplifications were performed for every patient. Results were considered positive when at least one of the amplified genome portions yielded a clear signal after hybridization.

RESULTS

Five of the 56 samples were positive (8.9%). Three patients were positive for both NS3 and NS5 amplifications. However, only one patient each was positive for NS3 or NS5a gene amplification. Virological results obtained for each type of vasculitis are summarized in Table I.

Four patients with HBV-related PAN were positive for GBV-C.

HBsAg was detected in 34% (19/56) of the cases. HCV serology was positive for two patients (3.6%). Three GBV-C-positive patients (two with HBV-PAN, one with MPA) had been transfused and two were IVDA (two HBV-PAN).

DISCUSSION

Among viruses recognized to be responsible for vasculitis, HBV was first described in PAN [1]. HCV is now a recognized aetiology for mixed cryoglobulinemia [5], but has rarely been observed in other systemic vasculitides [18]. HIV [19] and parvovirus B19 [6] have been described in rare cases of PAN. GBV-C, usually associated with HCV, has a high prevalence in blood donors and is frequently found in patients with type II mixed cryoglobulinemia [17]. A pathogenetic role for GBV-C is unknown and, in most patients, no disease is found. Nevertheless, due to the similarities between transmission routes for GBV-C, HBV and HCV, the responsibility of GBV-C needed to be investigated in vasculitis.

Our cohort of patients comprised two groups of PAN: one with HBV infection and the other without. Because of a recruitment bias, the group of patients with HBV-related PAN was over-represented and therefore increased the probability of detecting GBV-C. Sera from patients with different small-vessel vasculitides were also studied: their pathogeneses are related to antineutrophil cytoplasmic antibodies, but, to date, no aetiological factor has been identified and a virus could be a candidate.

Only five patients were positive for GBV-C RNA (8.9%); four simultaneously had HBV-related PAN and one MPA. Among them, the former four patients were at risk of acquiring a viral infection by contaminated syringes in the context of i.v. drug injection or transfusion, and the MPA patient had been transfused for gastric bleeding 1 month prior to virus testing.

Although the frequency of GBV-C was low in patients with most types of vasculitis, this value must be interpreted in light of the relatively high prevalence observed in French blood donors (4.2%) who had never been transfused [20]. Indeed, the rate can only be higher in our patient population exposed to the risk of virus transmission. Indeed, GBV-C was only

<p>| Table I |
|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Vasculitis</th>
<th>No. of patients</th>
<th>HBs Ag</th>
<th>Anti-HCV ELISA 2</th>
<th>GBV-C PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAN of unknown aetiology</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HBV-PAN</td>
<td>19</td>
<td>19</td>
<td>2 (10.5%)</td>
<td>4 (21%)</td>
</tr>
<tr>
<td>WG</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CSS</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MPA</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>1 (9%)</td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
<td>19 (34%)</td>
<td>2 (3.6%)</td>
<td>5 (8.9%)</td>
</tr>
</tbody>
</table>
detected more frequently in HBV-related PAN, a group comprised of patients at risk for blood or sexual transmission of the virus. This result is also comparable to the prevalence of GBV-C in immunocompromised patients who had received multiple packed transfusions (8.7%) [20]. Our results indicate that GBV-C does not play a primary role in the pathogenesis of small- and medium-vessel vasculitides.

REFERENCES