Coinfection with Multiple TT Virus Strains Belonging to Different Genotypes Is a Common Event in Healthy Brazilian Adults

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Testing of the DNA of TT virus (TTV) was done with serum samples obtained from 191 persons working in a public hospital of the city of Rio de Janeiro, Brazil. TTV DNA was detected by PCR in the sera of 125 (65.4%) individuals. PCR products were cloned, and sequences with a length of 159 bases surrounding the TATA signal region were determined for 100 clones derived from 31 individuals. One clone from each of 23 subjects was sequenced, while 7 to 19 clones from eight individuals were sequenced. None of the sera contained a viral sequence identical to that of any other individual. Phylogenetic analysis revealed the existence of a divergent TTV genotype possessing a single-base deletion at position 140. Among the eight persons for whom various sequences were analyzed, six were coinfected with between two and seven TTV strains belonging to different genotypes. The results suggest that coinfection with multiple TTV strains belonging to different genotypes is a common event in healthy Brazilian adults.

TT virus (TTV) is a newly discovered human virus that was first detected in the serum of a Japanese patient (initials, T.T.) with posttransfusion hepatitis (12, 13). The TTV genome is constituted by a single-stranded, circular DNA of negative polarity (9, 13). The TTV nucleotide sequence (3,818 to 3,853 nucleotides) does not show a significantly high homology to the sequence of any other virus. Several TTV isolates have been entirely sequenced (4, 6, 8, 9, 14), revealing a high degree of divergence among strains and the existence of at least 16 genotypes, which are separated by an evolutionary distance greater than 0.30 (15).

Although TTV DNA titers closely correlated with aminotransferase levels in the sera of some patients during posttransfusion hepatitis (12), no clear association between TTV infection and human liver disease has been established at this time. Very high prevalences (62 to 96%) of TTV infection have been found in healthy populations of Japan (15, 20) as well as in developing Asian, African, and South American countries (1, 11, 17).

As was initially demonstrated, TTV transmission occurs through the parenteral route (12). However, very high prevalences in healthy populations indicate the existence of other routes of transmission.

Coinfection with multiple TTV strains has been described for people exposed to blood and blood products (3, 5, 21), as well as for patients with liver disease (2, 7, 22). Here we show that such a coinfection is a common event in Brazilian health care workers and that a healthy person can be coinfected by at least seven strains.

**RESULTS**

Seroprevalence of TTV DNA. One hundred ninety-one health care workers (47 men, 144 women) of a public hospital of the city of Rio de Janeiro, Brazil, were enrolled in this study. Serum samples were analyzed for the presence of TTV DNA. One hundred twenty-five samples were positive, corresponding to a prevalence of 65.4%.

Phylogenetic analysis. PCR products were cloned, and sequences of 159 bases (nucleotides 26 to 184) surrounding the TATA signal region localized upstream of open reading frame 2 (ORF2) were determined for 100 clones derived from 31 individuals. One clone from each of 23 subjects was sequenced, while 7 to 19 clones were sequenced from eight individuals. A total of 59 different sequences was thus obtained.

Figure 1 shows a phylogenetic tree which includes these 59 sequences along with 10 sequences available in databases and belonging to different TTV genotypes. A large genetic diversity was observed among the isolates from this study. No two se-
quences were identical when derived from different persons. Evolutionary distances between our sequences were up to 0.49. A group of 14 sequences constituted a separate branch (Fig. 1). These had in common a 1-nucleotide deletion at position 140. The genetic distances between strains of this cluster were less than 0.30.

Despite the large genetic diversity observed here, some stretches of the genome were perfectly conserved in all sequences. This was notably the case for the TATAA motif at nucleotides 86 to 90 and an ATG codon (position 107) which had been initially proposed to be the translation initiation codon of ORF2 (13).

**Coinfection with multiple TTV isolates.** Several clones derived from the same serum were sequenced to determine if

![Phylogenetic tree of 69 TTV isolates.](image-url)
TABLE 1. Demographic, professional, serological, and clinical data of the subjects for whom several TTV clones were sequenced

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Age</th>
<th>Profession</th>
<th>Anti-HAV</th>
<th>HBsAg</th>
<th>Anti-HBs</th>
<th>Anti-HBc</th>
<th>Anti-HCV</th>
<th>ALT (IU/liter)</th>
<th>AST (IU/liter)</th>
<th>Blood transfusion</th>
<th>Surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC098</td>
<td>F</td>
<td>44</td>
<td>Assistant nurse</td>
<td>Pos</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>23</td>
<td>80</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>SC160</td>
<td>F</td>
<td>40</td>
<td>Assistant nurse</td>
<td>Pos</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>15</td>
<td>34</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>SC189</td>
<td>F</td>
<td>22</td>
<td>Medical student</td>
<td>Neg</td>
<td>Neg</td>
<td>Pos</td>
<td>Neg</td>
<td>Neg</td>
<td>16</td>
<td>16</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>SC314</td>
<td>F</td>
<td>43</td>
<td>Assistant nurse</td>
<td>Pos</td>
<td>Neg</td>
<td>Pos</td>
<td>Neg</td>
<td>Pos</td>
<td>11</td>
<td>13</td>
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<td>Yes</td>
</tr>
<tr>
<td>SC319</td>
<td>M</td>
<td>50</td>
<td>Assistant nurse</td>
<td>Pos</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>28</td>
<td>20</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>SC484</td>
<td>M</td>
<td>42</td>
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<td>Pos</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>36</td>
<td>34</td>
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<td>Yes</td>
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<tr>
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<td>F</td>
<td>45</td>
<td>Attendant</td>
<td>Pos</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>32</td>
<td>43</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
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<td>50</td>
<td>Assistant nurse</td>
<td>Pos</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>24</td>
<td>28</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

a HAV, hepatitis A virus; HCV, hepatitis C virus; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Mixed infections of TTV have been reported in individuals at high risk for infection with parenterally transmitted viruses, such as intravenous drug users (9), hemophiliacs (9, 21), and hemodialysis patients (3, 5), as well as in patients with liver disease (2, 7, 9, 22). Recently, coinfections with two or three TTV strains have been reported to be in some healthy Japanese individuals (15, 16). Here we show that such a mixed infection is a common event in healthy Brazilian people, at least in health care workers. Our results confirm and extend previous observations showing that infection by a given genotype is not protective against the superinfection by another type (5, 15). Furthermore, we show that the number of TTV isolates infecting an individual can be high. For example, for subject SC894, the nucleotide sequences of 19 clones were determined and 11 distinct sequences were obtained (Table 2). Genetic distances between two TTV sequences in the same serum could be very close or very divergent (up to 0.45 for subject SC314). Although the mutation rate of the TTV
FIG. 2. Alignment of partial nucleotide sequences of 36 TTV variants found in eight health care workers. The number of clones showing identical sequences is given. The sequence of prototype isolate TA278 of genotype 1a (13) is indicated at the top. Dashes represent the same nucleotides as in the TA278 isolate. In two clones from subject SC319, an insertion of an A nucleotide occurs at position 45; slashes indicate the absence of this nucleotide in the other sequences.


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REFERENCES


