Detection of sequences of TT virus, a novel DNA virus, in German patients

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Recently a novel DNA virus, tentatively designated TT virus (TTV), that possibly accounts for some of the cases of liver disease of unknown aetiology, was identified in Japanese patients. Using specific primer pairs for conserved regions, we detected TTV DNA by PCR in 16/84 (19%) German patients awaiting orthotopic liver transplantation because of decompensated liver cirrhosis (of diverse causes); in 4/25 (16%) patients with non-A–G hepatitis; in 1/7 patients with autoimmune hepatitis; and in one intravenous drug user. Sequence analysis showed that in contrast to the findings in Japanese patients only about 37% of our TTV sequences belonged to genomic group 1 but about 58% belonged to group 2, including several sequences belonging to a further subgroup tentatively designated group 2c. Further studies to clarify whether the novel virus has hepatitis-inducing capacity or other clinical significance are needed.

Recently a novel DNA virus, tentatively designated TT virus (TTV), was identified; it possibly accounts for some of the cases of acute and chronic liver disease of unknown aetiology (Nishizawa et al., 1997; Okamoto et al., 1998). TTV DNA was first isolated by representation analysis from serum of a patient (TT) with post-transfusion non-A–G hepatitis. At present, 3739 bases have been determined (accession no. AB008394) containing two possible open reading frames encoding 770 and 202 amino acids, respectively. TTV is reported to be an non-enveloped, single-stranded DNA virus with a density of 1.26 g/cm³ in sucrose. Although TTV has no significant similarity to sequences of known parvoviruses, a relationship to the family Parvoviridae has been discussed. In Japan, TTV DNA was detected in 47% (9/19) of patients with fulminant non-A–G hepatitis and in 46% (41/90) of patients with chronic liver disease of unknown aetiology (Okamoto et al., 1998). Furthermore, it was also detected in high rates in patients with parenteral risk-like haemophilia (19/28), intravenous drug users (14/35) and haemodialysis patients (26/57); 12% of Japanese blood donors (34/290) without any symptoms of disease were found to be TTV PCR positive. Sequence analysis of a 356 bp PCR product revealed considerable differences in nucleotide sequences from Japanese patients. They were classified into two genomic groups (G1 and G2) differing by more than 30% of their nucleotide and amino acid sequences and each was further divided into two subgroups differing by about 15%. Of the Japanese sequences 97% belonged to group 1 and only 3% to group 2. At present there are in GenBank a further eight partial TTV sequences from Japan (accession nos AB011486–AB011494) and one from China (accession no. AF055897) belonging to subgroups G1a and G1b.

In order to investigate the occurrence of TTV-like sequences in German patients, we designed specific primers for conserved regions within TTV genome groups 1 and 2 (Okamoto et al., 1998) and compared the nucleotide and the deduced amino acid sequences with those from Japan.

We examined 84 pretransplant sera of patients who had received orthotopic liver transplantation (OLT) because of decompensated liver cirrhosis (of diverse causes), as well as 25 sera from patients with chronic and acute liver disease of unknown aetiology, 7 with autoimmune hepatitis and 2 intravenous drug users. Nucleic acids were prepared from 140 µl serum by a spin column technique (Qiagen). One-tenth of the extracted DNA was then subjected to PCR in a 25 µl reaction mixture. For the first-round PCR, primer TT6 (sense: 5’ ACAGACAGAGGAAGGCAA) and primer TT7 (antisense: 5’ TCCAYTTAGCCTCATT, with Y = C and T) were used for the amplification of a product of 329 bp. The second-round PCR was carried out with TT8 (sense: 5’

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The sequences reported in this paper have been deposited in GenBank, accession nos AF060545–AF060550.
Fig. 1. Comparison of nucleotide sequences of TTV from patients treated in German hospitals with representative sequences of four Japanese subgroups (G1a: TA278; G1b: TX011; G2a: TS003; G2b: NA004) according to Okamoto et al. (1998). Numbering is according to GenBank accession no. AB008394. CH/1 is the Chinese isolate, GenBank accession no. AF055897. All others (B/1, B/2, BT/3-BT/73, L/1 to L/3 and P/1) are sequences from patients with various liver diseases (as described in the text) treated in German hospitals.
TTV detection in German patients by PCR

Fig. 2. Comparison of deduced amino acid sequences of TTV from patients treated in German hospitals (L/1 to L/3; B/1 and B/2; BT/3 to BT/73; as described in Fig. 1) with TTV sequences from Japan (G1a: TA278; G1b: TX011; G2a: TS003; G2b: NA004) according to Okamoto et al. (1998). CH/1 represents the sequence of the Chinese isolate, GenBank accession no. AF055897.

AACATGYTATGGATAGACTGG, with Y = C and T) and TTV (antisense: 5'-CGGCATTTACTACCTTTCCA), yielding an amplicon of 267 bp. Amplification was performed for 35 cycles (94 °C 30 s, 42 °C 30 s, 72 °C 45 s; for the last 3 min extension at 72 °C) and the nested PCR products were sequenced directly using an ABI Prism 377 DNA sequencer (Perkin-Elmer). Nucleotide sequences were aligned with the CLUSTAL W program version 1.6 (Thompson et al., 1994). Phylogenetic trees were produced using the Phylogeny Interference Package (PHYLIP) version 3.57c (Felsenstein et al., 1989). Evolutionary distances were estimated using the DNADIST program and unrooted phylogenetic trees were constructed using the NEIGHBOR program (PHYLIP). Analysis of 100 bootstrap re-samples of the nucleotide alignment data sets was performed using the SEQBOOT and CONSENSE programs of PHYLIP.

With our nested primer pairs, TTV sequences were detected in 425 patients (B/1, B/2, L/2, P/1) with non-A–G hepatitis (16%), in 17 (14%) patients (L/3) with autoimmune hepatitis and in 1/2 intravenous drug users (L/3, who was coinfected with hepatitis B, C and delta viruses). Furthermore, TTV DNA was detected in 16/84 (19%) patients (L/3) before OLT. Of these 9 (about 60%) had hepatitis virus-induced cirrhosis (HCV: BT/4, BT/13, BT/51, BT/62, BT/64, BT/67; HBV: BT/60; HBV and HCV: BT/73; HBV and HDV: BT/43). The remaining patients had primary biliary cirrhosis (BT/10, BT/38), primary sclerosing cholangitis (BT/3), Caroli syndrome (BT/78) and alcoholic liver disease (BT/37, BT/47); one patient was retransplanted due to ischaemic-type biliary lesion (BT/59).

Our further investigations will deal with long-term follow-ups after OLT in order to compare the replication behaviour of TTV with that observed in OLT patients with hepatitis C or G virus infection (Berg et al., 1996; Fukomoto et al., 1996) and to check the hepatitis-inducing activity of the novel virus. Okamoto et al. (1998) showed that TTV DNA is detectable in liver tissues of patients in titres equal or to 10–100 times higher than those in the corresponding sera.

Nineteen sequences of 227 bp (nt 2139–2365, primer sequences excluded) were analysed; representative sequences have been submitted to GenBank, accession nos AF060545–AF060550. The comparison with each other and with sequences representative of four different subgroups published by Okamoto et al. (1998) (Figs 1 and 2) revealed that six sequences belong to subgroup G1b with an intratypical identity of 91 ± 7–97 ± 4% (91–96% at the amino acid level). One sequence (BT/59) seems to belong to group 1 but shows 19 ± 8% and 14 ± 5% nucleotide differences compared with subgroups 1a and 1b, respectively. Two of our sequences belonged to subgroup G2b (95 ± 2–96 ± 5% nucleotide identity, 97 ± 4–98 ± 7% amino acid identity), and nine to genome group G2 [intratypical identity 92 ± 2–95 ± 2% (nucleotide), 88 ± 2–96 ± 1% (amino acid)] but show about 15–16% nucleotide (8–15% amino acid) when compared with sequences of subgroups G2a and G2b. Thus, a further subgroup tentatively designated G2c had to be established (Fig. 3). The sequence from a patient with non-A–G fulminant liver disease (P/1) showed 22 ± 4–29 ± 8%
nucleotide differences when compared with subgroup 1a and 1b sequences and 15.8–23.7% differences compared with subgroup 2a, b and c sequences, respectively, and seems to be a representative of a further subgroup or new genomic group.

We have shown the occurrence of TTV sequences in a selection of patients with liver diseases treated in German hospitals. Comparison of nucleotide and deduced amino acid sequences showed considerable divergence leading to their classification into different genomic subgroups. In contrast to the distribution of Japanese sequences, 36.8% of our sequences belonged to group 1 and 57.9% to group 2. One patient seems to represent a new genomic group or subgroup. We detected TTV DNA both in patients with cryptogenic liver disease and in patients coinfected with other hepatitis-inducing viruses, but at lower rates than published by Okamoto et al. (1998). Considering the high prevalence of TTV DNA in Japanese blood donors (12%), further studies are needed to clarify whether the novel DNA virus has hepatitis-inducing capacity or represents only one more innocent bystander, like hepatitis G virus.

References


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