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A cDNA fragment of hepatitis C virus isolated from an implicated donor of post-transfusion non-A, non-B hepatitis in Japan

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ABSTRACT

Recently, a cDNA from the hepatitis C virus (HCV) RNA genome has been isolated in the USA from a chronically infected chimpanzee. In order to isolate HCV cDNA derived from human material, RNA was extracted from plasma of a Japanese blood donor implicated in post-transfusion non-A, non-B hepatitis and HCV cDNA was synthesized and amplified by the PCR method using HCV-specific oligonucleotide primers. The cDNA fragment, 583 nucleotides long, showed 79.8% homology at the nucleotide level and 92.2% homology at the amino acid level compared with the prototype HCV cDNA. These results provides further evidence to show that HCV is closely associated with the development of post transfusion non-A, non-B hepatitis.

INTRODUCTION

A cDNA of the hepatitis C virus (HCV) genome has recently been cloned from an infected chimpanzee which developed non-A, non-B hepatitis following inoculation with a contaminated human factor VIII concentrate (1). HCV is a member of the positive-strand RNA viruses, related to either the flaviviridae or togaviridae (1). Seroepidemiological studies based on the detection of antibody against a recombinant HCV antigen (C100-3) suggest that HCV is associated with most posttransfusion non-A, non-B hepatitis (PTNANBH) cases around the world, including Japan (2). Further examination has shown that anti-C100-3 antibody detected by this assay seems closely associated with the infectivity of HCV, because most blood tranfusions resulting in the development of PTNANBH have been shown to contain at least one unit of antibody-positive blood (3).

To examine whether antibody-positive blood does indeed contain HCV virions, we have attempted to directly isolate HCV cDNA derived from plasma of an implicated blood donor and have investigated the degree of homology with the original chimpamzee isolate.

MATERIALS AND METHODS

cDNA synthesis and PCR amplification

HCV virions in plasma of an implicated donor were pelleted by ultracentrifugation according to the procedure described previously (4) and RNA was extracted by the guanidinium/cesium chloride method (5). The RNA was further purified by phenol/chloroform extraction in the presence of urea (6). RNA obtained from 1ml of plasma was used for cDNA synthesis with both 10 units of reverse transcriptase (BioRad) and 1 µg of an antisense primer 526A, 5'-GACATGCATGTCATGATGTA-3', by the method described previously (7). The cDNA was then amplified by polymerase chain reaction (PCR) using the standard method (8) after adding 1 µg of a sense primer 464S, 5'-GGCTATACCGGCGACTTCGA-3'. These primers were designed from the sequence of the original HCV chimpanzee isolate (Q-L. Choo et al. manuscript in preparation).

Cloning and sequencing

The cDNA fragment amplified by the PCR method was visualized by ethidium bromide staining. A DNA band of the expected size was exised from the gel, purified and cloned by blunt-end ligation into the SmaI site of pUC119 (9). Three independent clones were isolated and their nucleotide sequences were determined by dideoxy chain termination method.

RESULTS

Isolation of HCV cDNA derived from an implicated donor

A Japanese male patient developed typical PTNANBH after receiving six units (1 unit is 200 ml) of blood transfusion. Samples of the blood donations were retrospectively assayed for the C100-3 antibody and one donor blood sample was found to contain HCV antibody. The plasma of the implicated donor was used as a starting material for cDNA cloning. cDNA was synthesized using the RNA extracted from the plasma as template with the synthetic oligonucleotide primer 526A, which is specific to the putative non-structural region 3 (NS3) of the prototype HCV (Choo et al., manuscript in preparation). The cDNA was then amplified by PCR method after adding the primer 464S, the 3' end of which is 583 nucleotides (nt) upstream from the 3' end of the primer 526A in the prototype HCV genome. A single band of the expected size (about 620 nt) was visible following agarose gel electrophoresis by ethidium bromide staining after 30 cycles of PCR reactions. Three independent clones inserted into the plasmid vector were isolated and their nucleotide sequences were determined.

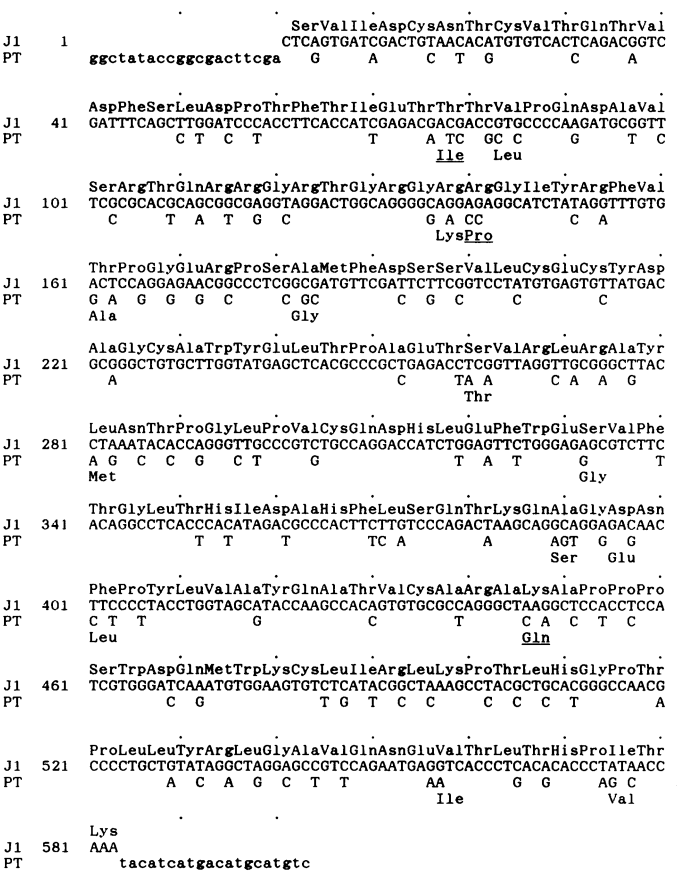

Figure 1: Nucleotide sequence of HCV cDNA J1 and the deduced amino acid sequence. J1, HCV cDNA isolated from human material; PT, prototype HCV cDNA. Sequences of primers are shown in small letters. Numbers represent the nucleotide positions after the 3' end of the sense-strand primer. For prototype HCV cDNA, only the nucleotides and amino acids different from J1 cDNA are shown. An underline of amino acid sequence under the prototype amino acid shows that the amino acid is not only different but also unrelated to that of J1 cDNA.

Sequence analysis of the HCV cDNA

The nucleotide lengths of these three cDNA clones were identical (623 bp), but there were five nucleotide differences (see Discussion). Therefore, we constructed a consensus sequence (named J1) of the three clones and compared the J1 sequence with the sequences of the chimpanzee derived HCV cDNA (PT for prototype) (Fig. 1). The nucleotide homology between J1 and PT is 79.8%

Table 1: Base-level heterogeneity of HCV cDNA derived from a single healthy carrier

	differences (J1 / clone)		
clone	position	nucleotide	amino acid
pU1-4652c	59	ACC / GCC	Thr / Ala
pU1-4652d	perfectly identical to J1 sequence		
pU1-4652e	226	GGC / GGA	Gly(unchanged)
	451	GCT / GCC	Ala(unchanged)
	515	CCA / GCA	Pro / Ala
	535	AGG / AGA	Arg(unchanged)

Underline, different nucleotides.

(465/583 nt), while the amino acid homology is higher (92.2%, 179/194 amino acids). When amino acid homology was analysed in such a way that functionally similar amino acids are regarded as homologous, the homology reaches 98.5% (191/194 amino acids). Therefore, the sequence of the Japanese HCV cDNA significantly different from the original isolate at the nucleotide level but it is highly homologous at the amino acid level.

We observed five nucleotide differences among the three independent cDNA clones (Table 1). These differences may be explained as artifacts of the PCR amplification (8) or they may reflect viral heterogeneity: out of these five different nucleotides, three fall on the third letters of the codons and do not change the amino acid. Both of the remaining two different nucleotides fall on the first letter of the codons and change amino acids from Thr to Ala, and from Pro to Ala, all of which are small, neutral amino acid residues. These results strongly suggest that the observed nucleotide differences reflect genuine viral genome heterogeneity.

DISCUSSION

We have shown that the HCV genome can be detected in plasma from an implicated blood donor identified by the HCV C100-3 antibody assay. This result provides further evidence that the anti-C100-3 antibody is associated with HCV infectivity, and confirms the rationale of the usefulness of this antibody test in blood screening.

The HCV cDNA isolated here showed only 79.8% homology with the

prototype HCV cDNA sequence. As a result, the two cDNAs do not hybridize stably under stringent washing conditions and it was necessary to try several different primers to isolate the new cDNA. In spite of the low nucleotide homology, the amino acid sequences are highly conserved. Surprisingly, out of 118 different nucleotides, 103 (87.3%) are silent mutations. Moreover, out of 15 amino acid differences, 11 are changes between related amino acids, such as Ile to Val, etc. (Fig. 1). The result is consistent with our previous seroepidemiological studies which have shown that the recombinant C100-3 antibody assay derived from the prototype HCV cDNA can detect most cases of PTNANBH in USA, Japan and Italy.

Although the C100-3 antibody detection system has been developed, a routine assay for viral antigen has not yet been developed. Therefore, the detection of the HCV genome by cDNA synthesis and PCR amplification probably represents a valuable direct assay for the HCV virions in near future.

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