Expression of the amino-terminal half of the NS1 region of the hepatitis C virus genome and detection of an antibody to the expressed protein in patients with liver diseases

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A cDNA fragment encompassing the 5'-terminal half of the NS1 region of the hepatitis C virus (HCV) genome was cloned. The cDNA was expressed in insect cells using a recombinant baculovirus, and a protein band of approximately 21K was identified by immunoblotting with a serum sample from a patient with chronic hepatitis C. Antibody to the protein was detected in sera from 13.4% of patients with chronic non-A, non-B hepatitis (NANBH), 20.8% of patients with liver cirrhosis and 16.8% of patients with hepatocellular carcinoma with no serum markers for hepatitis B virus infection. However, the antibody was not detected in sera from patients with acute NANBH. The prevalence of antibody to the protein encoded by the NS1 region was lower than that of antibody to the HCV core protein, but much higher than that of antibody to the envelope protein. Thus, the NS1 region of the HCV genome is suggested to encode a protein produced during the course of HCV replication.

Hepatitis C virus (HCV) is a major causative agent of non-A, non-B hepatitis (NANBH) world-wide (Choo et al., 1989; Kuo et al., 1989; Alter et al., 1989; Miyamura et al., 1990). The virus genome is a positive-stranded RNA of about 9.4 kb (Kato et al., 1990; Takamizawa et al., 1991; Chop et al., 1991). The genome organization has been found to be very similar to that of flaviviruses or pestiviruses (Choo et al., 1990; Miller & Purcell, 1990; Takeuchi et al., 1990; Han et al., 1991), and as is the case for these viruses, the HCV genome is thought to encode a large precursor polyprotein from which virus-specific proteins are processed by either host or viral proteases (Houghton et al., 1991). In view of the similarity among these three viruses, the first non-structural protein (NS1) may play an important role in virus replication (Rice et al., 1986). Antibody to the protein translated from the NS1 region of the yellow fever virus genome has been shown to have protecting activity (Cane & Gould, 1988; Schlesinger et al., 1986). In this study, we cloned a cDNA fragment encompassing the 5'-terminal portion of the NS1 region of the HCV genome and expressed it in insect cells using a recombinant baculovirus. Antibody to the protein expressed was detected in sera from patients with NANBH.

A sample of plasma (1 ml) from a blood donor whose blood had been regarded to be implicated in post-transfusion NANBH was centrifuged at 15850 g for 15 min (Bradley et al., 1985). RNA was extracted by the guanidinium/caesium chloride method (Maniatis et al., 1982) and purified by phenol/chloroform extraction with an equal volume of 7 M-urea (Berk et al., 1979).

The RNA was used to synthesize cDNA by using 10 units of reverse transcriptase and an antisense primer (5' AATCCAGTTGAGTTCATCCA 3') (150 pmol/μl). The cDNA was then amplified by the polymerase chain reaction (PCR) method (Saiki et al., 1985; Suzuki et al., 1989) after adding a sense primer (5' ACTGCCCTGAACTGCAATGA 3'). These primer sequences were deduced from nucleotide sequences of the putative NS1 region published previously (Kato et al., 1990; Choo et al., 1991). The amplified cDNA fragment was purified by agarose gel electrophoresis and cloned into the SnaBI site of charomid SB-42 (S. Boonmar & I. Saito, unpublished results) by blunt-end ligation. Three independent clones were isolated, and their nucleotide sequences were determined by the dideoxynucleotide chain termination method (Sanger et al., 1977) and found to be identical (K. Takeuchi et al., unpublished results). The
cDNA was ligated to the 3' terminus of pSR820x (Matsuura et al., 1992), which contains the junction between the envelope and NS1 regions, and then digested with HincII. The cDNA fragments of 531 bp were considered to encode the N-terminal half of the NS1 protein (amino acids 382 to 559; Kato et al., 1990; Takeuchi et al., 1990). This cDNA fragment was then inserted into the SpeI site of a cassette plasmid derived from baculovirus cDNA, pAcYM1 (Matsuura et al., 1989). The resultant recombinant DNA (pAc1520) was transferred into a baculovirus, Autographa californica nuclear polyhedrosis virus (AcNPV), by cotransfection of pAc1520 and wild-type AcNPV DNA into insect (Spodoptera frugiperda) cells (Sf-9 cells). A recombinant baculovirus, Ac1520, was obtained by homologous recombination, grown and plaque-purified.

Cell lysates were prepared from Sf-9 cell cultures infected with either Ac1520 or AcNPV by the procedure described previously (Chiba et al., 1991). The lysate was separated on a 15% SDS–polyacrylamide gel and its reactivity with a sample of serum from a hepatitis C patient was examined by immunoblotting as described previously (Harada et al., 1991; Matsuura et al., 1992). The patient had been clinically diagnosed as having hepatitis C and the HCV infection was confirmed by the presence of anti-C100 (Kuo et al., 1989) and anti-p22 (HCV core protein) (Harada et al., 1991) antibodies, as well as by the detection of HCV RNA in the plasma by the reverse transcriptase PCR method. As shown in Fig. 1, a band at about 21K was detected (N1520). Faint bands that migrated more slowly were also observed in wild-type AcNPV-infected cells. Since the HCV cDNA fragment used in this experiment lacked a possible signal sequence for the translocation of the protein into the endoplasmic reticulum (Takeuchi et al., 1990), the protein detected, N1520, was considered not to be glycosylated despite the presence of six possible glycosylation signals in this region of the HCV genome. An unglycosylated protein of about 180 amino acids may correspond to the band of about 21K.

Since serum from a patient with typical chronic hepatitis C reacted with the expressed N1520 protein, we examined 463 serum samples from NANBH patients for the presence of antibody to N1520 by immunoblotting. Of 53 cases with acute NANBH, 14 were confirmed to have hepatitis C owing to a positive reaction for anti-C100 and anti-p22 antibodies. Of 141 patients with chronic hepatitis, 144 with liver cirrhosis and 125 with hepatocellular carcinoma, 86, 103 and 107, respectively, had anti-HCV markers. The prevalence of antibody to the N1520 protein among these patients is summarized in Table 1. Although anti-C100 or anti-p22 antibody reactivity alone is not regarded as sufficient evidence for HCV infection, it is noteworthy that all sera reactive with N1520 protein had either anti-C100 or anti-p22 antibodies, or both.

Although the overall rate of positive reactions for anti-N1520 antibody among patients with non-A, non-B liver diseases was lower than that of anti-C100 or anti-p22 antibodies (Kuo et al., 1989; Watanabe et al., 1991), it was higher than that of antibody to HCV envelope protein (Table 1). The latter protein is expressed similarly in insect cells by a recombinant baculovirus vector (Matsuura et al., 1992). All the patients who had anti-envelope protein antibody were also positive for anti-N1520 antibody. Furthermore, in one patient who was followed throughout the course of HCV infection, anti-N1520 antibody was detectable 2 months prior to the detection of anti-envelope protein antibody.
These observations indicate that a protein antigenically identical to the N1520 protein is present in hepatitis C patients and that some patients develop antibody to it. The low frequency of detection of this antibody in NANBH patients in this study may be due to several reasons. (i) In the immunoblotting assays used to detect anti-N1520, antigen was denatured by SDS. The procedure may have destroyed some conformational epitopes and missed some antibodies which react only with the native form of the NS1 protein structure. Using the anti-N1520 antibody-positive patient sera described above, we could stain the cytoplasm of Ac1520-infected cells by immunofluorescence (Fig. 2). However, only sera shown to react with N1520 by immunoblotting were positive by immunofluorescence. (ii) We used the 5'-terminal half of the putative NS1 region and expressed N1520 was not glycosylated. There might be antibodies which react only with complete NS1 protein. (iii) The nucleotide sequence of the NS1 region of the HCV genome is variable among different HCV isolates, and two hypervariable domains are located within the region employed in this study (Weiner et al., 1990; Hijikata et al., 1991). The similarity between our clone and other Japanese type 1 isolates is only 83% at the amino acid level. Recently, the existence of type 2 HCV has been reported (Enomoto et al., 1990; Tsukiyama-Kohara et al., 1991; Okamoto et al., 1991). The similarity between our clone and other Japanese type 1 isolates is only 83% at the amino acid level. Recently, the existence of type 2 HCV has been reported (Enomoto et al., 1990; Tsukiyama-Kohara et al., 1991; Okamoto et al., 1991). The similarity between HCV type 1 and type 2 in the NS1 region is lower (71 to 73%) than that between different type 1 isolates (78 to 83%). Those patients infected with type 2 HCV may not react with the NS1 protein expressed by the type 1 HCV genome.

Nevertheless, the presence of anti-N1520 antibody in sera from patients with hepatitis C suggests that the nonstructural glycoprotein NS1 is synthesized during the course of HCV replication. NS1 has been detected on the surface of flavivirus-infected cells (Stohlman et al., 1975; Cardiff & Lund, 1976; Gould et al., 1985). Although its function is unknown, antibodies to the purified NS1 protein have been shown to protect immunized mice and rhesus monkeys against a challenge with yellow fever virus (Schlesinger et al., 1985) or with dengue virus (Schlesinger et al., 1987). One-quarter of the yellow fever virus unglycosylated NS1 protein expressed in Escherichia coli has also been shown to be protective (Cane & Gould, 1988). As anti-N1520 antibody was detected in sera from patients chronically infected with HCV, the anti-NS1 antibody may not be directly associated with neutralizing activity.

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References


Table 1. Detection of anti-N1520 antibody in sera from patients with non-A, non-B liver diseases and normal healthy blood donors

<table>
<thead>
<tr>
<th></th>
<th>No. examined</th>
<th>Hepatitis C*</th>
<th>Anti-envelope†</th>
<th>Anti-N1520</th>
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<tr>
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<td>2</td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Either anti-C100 or anti-p22 antibody (or both) was positive by ELISA (Kuo et al., 1989; Chiba et al., 1991).
† Anti-envelope protein antibody was detected by Western blotting (Matsuura et al., 1992).


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