Expression of HCV E2/NS1 protein as a fusion protein with maltose binding protein: detection of anti-E2/NS1 antibody in chronic liver disease

O Yokosuka, M Omata, Y Ito, M Ohto

Abstract
The presence of anti-E2 antibody was investigated in the serum samples of 46 patients with liver disease, who were positive for hepatitis C virus-RNA, and in five subjects HCV-RNA-negative acting as controls. Antibody to E2/NS1 protein was found in seven of 46 (15%) of the patients with liver disease but in none of the control subjects. In one patient who was treated successfully with interferon, the levels of anti-E2 gradually decreased and then finally disappeared after treatment. This suggests that the E2/NS1 protein may play a role in active viral replication.

(Hepatitis C virus (HCV) infection is a major cause of non-A, non-B liver disease. Recent studies on HCV have shown that the structural proteins are encoded near its amino terminal end. E2/NS1 protein is thought to be located between the envelope and non-structural proteins.1 2 We have expressed E2/NS1 protein as a fusion protein with maltose binding protein and investigated the presence of anti-E2/NS1 antibody in the serum samples of patients with liver disease.

Methods
Extraction of nucleic acids from HCV-RNA positive serum was followed by reverse transcription to cDNA. The E2/NS1 protein encoding sequence was then amplified using nested polymerase chain reaction. The amplified DNA sequence was ligated to the p-Mal-C expression vector3 (Figure) and transfected to Escherichia coli TBI. The E. coli clone with the ligated vector was then cultured in the presence of IPTG. The expressed E2/NS1 protein was added to SDS-PAGE (12.5% concentration) and transferred to a nitrocellulose filter. The nitrocellulose filter was incubated with 100-fold diluted original serum and the signal was detected by the immunoperoxidase method.

By expressing this E2/NS1 protein, serum samples from 46 patients with liver disease positive for HCV-RNA (five with acute hepatitis, 12 with chronic persistent hepatitis, 25 with chronic active hepatitis, and four with cirrhosis) and five subjects HCV-RNA negative were examined for the presence of anti-E2 antibody by western blot analysis.4

Results
By expressing the E2/NS1 sequence (339 amino acids) as a fusion protein with maltose binding protein, about 80 KD of protein (including 38 KD E2/NS1 protein) which reacted with patient’s serum was obtained. Using this protein as antigen, the antibody to E2/NS1 protein was detected in seven of 46 (15%) patients with HCV related liver disease (Table). It was not detectable in any of the five normal subjects. Serial serum samples were also taken from a patient with hepatitis C who had been treated successfully with interferon alfa (24 million units per day for 28 days). In this patient, anti-E2 antibody was detected

Number of patients testing positive for anti-E2 antibody

<table>
<thead>
<tr>
<th>Patients/subjects tested (n)</th>
<th>Patients/subjects with antibody to E2/NS1 protein (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCV-RNA positive patients</td>
<td></td>
</tr>
<tr>
<td>Acute hepatitis</td>
<td>5 0</td>
</tr>
<tr>
<td>Chronic persistent hepatitis</td>
<td>12 2</td>
</tr>
<tr>
<td>Chronic active hepatitis</td>
<td>25 4</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>4 2</td>
</tr>
<tr>
<td>Total</td>
<td>46 7 (15%)</td>
</tr>
<tr>
<td>HCV-RNA negative normal subjects</td>
<td>5 0</td>
</tr>
</tbody>
</table>

Figure Schematic representation of the E2/NS1 protein expression vector.
Detection of anti-E2/NS1 in chronic liver disease

before interferon treatment, but then gradually decreased and finally disappeared after successful treatment.

Conclusions

The biological role of the E2/NS1 protein is unknown. By analogy with other flaviviruses, however, it is thought to play a role in the formation of viral membranes. The detection of anti-E2 antibody in patients with chronic hepatitis C indicates that this is not a neutralising antibody. Moreover, the disappearance of the antibody during interferon treatment suggests that this protein is related to active viral replication.

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