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

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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.

(Gut 1993; supplement: S64-S65)

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 ITPG. 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.

Using 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

Figure Schematic representation of the E2/NS1 protein expression vector.

Number of patients testing positive for anti-E2 antibody

Patients/subjects tested (n)	Patients/subjects with antibody to E2/NS1 protein (n)	
HCV-RNA positive patients		
Acute hepatitis	5	0
Chronic persistent hepatitis	12	2
Chronic active hepatitis	25	3
Cirrhosis	4	2
Total	46	7 (15%)
HCV-RNA negative normal subjects	5	0

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

treatment suggests that this protein is related to active viral replication.

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

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