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Detection of Hepatitis C Virus Infection by Enzyme-linked Immunosorbent Assay System Using Core Protein Expressed in *Escherichia coli*

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Enzyme-linked immunosorbent assay of the core protein of hepatitis C virus (HCV) expressed in *E. coli* led to detection of the antibody against this virus in patients with chronic hepatitis. Some of the negative results obtained using a different viral protein became positive with this *E. coli*-expressed viral protein, and were also positive for the viral RNA. Thus, use of the core protein of HCV facilitates accurate detection of HCV infection.

Key words: Hepatitis C virus — Hepatitis — ELISA — Serodiagnosis

Many cases of blood-transfused non-A, non-B hepatitis are linked to infectious agent(s). Part of the gene for one of these agents was molecularly cloned and the agent was termed hepatitis C virus (HCV).[1] A serodiagnostic assay system was developed to screen HCV infection, using a non-structural protein, C-100 synthesized in yeast.[2] Although this system was used in epidemiological studies, screening and other HCV-related investigations, false-positive cases of the virus infection have been reported.[3,4] In addition, there were cases of negative results obtained with this antibody assay system, even though positive results were obtained for virus infection in the detection of HCV RNA using polymerase chain reaction.[5]

In previous experiments, we expressed a putative viral core protein in *E. coli* and numerous chronic hepatitis cases negative for the anti C-100 antibody were shown to be positive in western blot assays using the core protein.[6] Since most of these cases were also positive for HCV RNA, assay of the virus using the core protein is more accurate than that using C-100 for examining cases of chronic hepatitis.

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Abbreviations used: HCV, hepatitis C virus; ELISA, enzyme-linked immunosorbent assay; RT-PCR, reverse transcription followed by polymerase chain reaction.

We developed an enzyme-linked immunosorbent assay system (ELISA) to detect HCV at either early or late stages of infection. This system is expected to be useful for serodiagnosis of HCV infection.

E. coli lysate which contains the core protein of HCV was obtained, as described.[6] A 40% saturated ammonium sulfate precipitate of the lysate was dissolved in a buffer of 50 m*M* Tris-HCl (pH 8.0)-1 m*M* EDTA-14 m*M* β-mercaptoethanol containing 7 *M* urea and applied to a DEAE cellulose column (1 cm diameter, 10 ml). The pass-through fraction of the column was further applied to a phosphocellulose (p11) column (1 cm diameter, 10 ml). The column was eluted with a linear gradient from 0 to 0.5 *M* KCl-50 m*M* phosphate buffer (pH 6.8)-14 m*M* β-mercaptoethanol-7 *M* urea. The eluate with 0.4 *M* KCl was applied to a hydroxyapatite column (1 cm diameter, 5 ml). The column was eluted with a linear gradient from 50 m*M* to 0.5 *M* NaCl in phosphate buffer (pH 6.8)-β-mercaptoethanol-7 *M* urea. Each fraction was pooled and fractions in which the core protein was eluted were collected. These procedures produced a single major band of the core protein, detected by staining with Coomassie Brilliant Blue (Fig. 1). The protein was dialyzed, after addition of sodium dodecyl sulfate (SDS) to a final concentration of 0.1%, against phosphate-buffered saline (PBS) containing 0.1% SDS. Each well of an ELISA plate was coated with about 25 ng of the protein and 10% bovine serum albumin-10% skim milk was used for blocking. Ten μl of serum was added to each

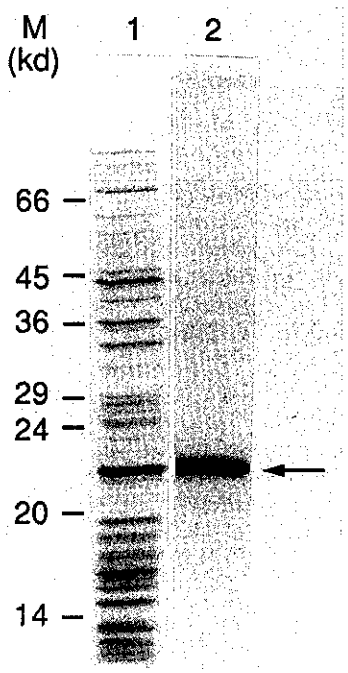

Fig. 1. Purification of a core protein expressed in *E. coli*. Lane 1, total protein of *E. coli* after induction by isopropylthio-β-D-galactoside. Lane 2, purified core protein stained with Coomassie Brilliant Blue. An arrow points to the core protein.

well and the antibody attached to the coated protein was allowed to react with peroxidase-coupled goat anti-human IgG. The enzyme reaction was carried out with *o*-phenylenediamine as the substrate in the presence of hydrogen peroxide and the enzyme activity was measured by reading the optical density at 490 nm.

The cut-off value for the ELISA test was assessed from values of the optical densities of 10 positive and 50 negative controls. Fifty sera were selected as negative controls from healthy persons with no elevated alanine aminotransferase and a negative assay for antibody against C-100, and 10 sera were selected as positive controls from patients with chronic hepatitis, having elevated alanine aminotransferase and being positive for antibodies against C-100. ELISA assay for those sera was carried out. The values of optical density at 490 nm for negative sera were lower than 0.10 and the values for positive sera were higher than 0.55. Then 200 sera without elevated alanine aminotransferase and negative for the antibody against core protein of HCV by western blot analysis, obtained at mass health examination, were analyzed by this ELISA method. All values read by this assay were lower than 0.150 except for two cases which gave values of 0.3 and 0.31. Therefore, the average value of 3 negative controls plus 0.300 was adopted as the cut-off value in this experiment. All judgments based on this ELISA assay were consistent with those obtained by western blot analysis.[6]

Table I. Immunoscreening for HCV Infection

		Anti-core antibody	
		+	−
Chronic hepatitis			
Anti-C-100	+	139	2
antibody	−	39	55
Acute phase of hepatitis			
Anti-C-100	+	5	5
antibody	−	7	9

The results of analyses of HCV infection for patients with non-A, non-B chronic hepatitis (CH) and for patients with acute-phase non-A, non-B hepatitis (AH) are shown in Table I. Diagnostic criteria followed the proposal by the Japanese Gastroenterology Society in 1979. Acute-phase non-A, non-B hepatitis indicates hepatitis with a liver inflammation lasting for less than 6 months. All patients with acute-phase non-A, non-B hepatitis were diagnosed as having chronic hepatitis afterwards. Finally, 141 (63%) and 178 (79%) of 225 Japanese patients with chronic hepatitis were found to be positive for the anti-C-100 antibody and the anti-core protein antibody, respectively. Only 2 were positive for the antibody against C-100 but negative for the antibody against the core protein. However, 39 were negative for the anti-C-100 antibody but positive for the anti-core protein antibody.

All samples giving inconsistent results were further analyzed for HCV RNA by reverse transcription followed by polymerase chain reaction (RT-PCR). The reaction conditions were the same as those previously described,[5] except for the set of primers used. The sequence of the primer used for reverse transcription was 5′-GTGCTCATGGTGCACGGTCTA-3′ (complementary to the sequence from nt317 to nt327[7]). For PCR, a primer of 5′-AGAGCCATAGTGGTCTGCGG-3′ (from nt122 to nt141[7]) was used in addition to the above primer. The region examined by PCR is the most highly conserved among HCV. Thus, the possibility of not detecting HCV owing to a mismatch of the primers and HCV RNA is greatly diminished. Two cases of chronic hepatitis which were positive for anti-C-100 antibody but negative for the anti-core protein were negative for HCV RNA, as determined by using this method (data not shown). However, 80% of those which were negative for the anti-C-100 but positive for the anti-core protein antibody were positive for the RNA (data not shown).

Table II. Detection of Antibodies against C-100 or Core in Patients with Acute Phase of Non-A, Non-B Hepatitis

Patient	BT[a]		0[b]	2	4	6	8	10 mo
A	+	C100		−	−			
		Core		+	+			
B	+	C100			+	+	+	
		Core			−	−	−	
C	+	C100		−	−	+	−	−
		Core		−	−	+	+	+
D	−	C100	+			+		
		Core	−			−		
E	−	C100	−	+	+	+	+	
		Core	+	+	+	+	+	
F	+	C100	−	+				
		Core	−	−				
G	−	C100	−	−				
		Core	+	+				

a) +means patients with a history of blood transfusion (BT).
b) Zero indicates the time of the first blood transfusion or the time of the first visit to a clinic, because of hepatic disease. mo, month. Patients A, B, C and F had elevated alanine aminotransferase activity from the time when the first examination of their blood for HCV antibodies was conducted, as indicated in this table.

Negative cases of HCV RNA among those with inconsistent results means either false-negative results or the prolonged presence of the antibodies after apparent eradication of the virus. The failure to amplify the virus RNA by PCR would also need to be ruled out.

Fewer than 50% of the patients were positive in both assay systems, at the acute phase of hepatitis (Table I). Ten (38%) and 12 (46%) of 26 cases were positive for C-100 and the core protein, respectively. Only 5 (19%) were positive in both these analyses. It is likely that the first appearance of each antibody differs from patient to patient in the early stage of the virus infection but gradually both antibodies become detectable, in the same individual, at the late stage of chronic-phase hepatitis.

Seven patients with early hepatitis were tested for these antibodies (Table II). Four of the 7 had a history of blood transfusion. Sera collected from 2 to 10 months after the first transfusion for those with a history of blood transfusion, or after the first visit to an out-patient clinic because of a hepatic disorder, where there was no history of blood transfusion, were tested for these antibodies. Antibody against C-100 was detected in some of the patients earlier than that against the core protein, yet in other patients, the result was the opposite.

Thus, C-100 alone or the core protein alone is insufficient to allow efficient detection of the early stage of HCV infection. Use of both proteins or proteins encoded by other regions of HCV may facilitate more accurate detection of this virus.

An assay system for IgM type antibody against core protein should be developed in order to detect the early stage of HCV infection. As treatment of HCV infectants with interferon alpha at an early stage of the infection increases the cure rate,[8] the early detection of HCV infection is an urgent problem.

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