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Transition of Antibody to Hepatitis C Virus from Chronic Hepatitis to Hepatocellular Carcinoma

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Fifty-eight patients with chronic hepatitis C were followed for more than 7 years. Of them, 10 patients were found to develop hepatocellular carcinoma, 14 to develop liver cirrhosis, 30 to sustain chronic hepatitis, and 4 to show subsidence of hepatitis. Antibody to hepatitis C virus (anti-HCV) disappeared from the 4 patients whose hepatitis subsided, but it persisted in the remaining 54 patients. The mean titer of anti-HCV was almost the same at the stages of chronic hepatitis and of cancer in the 10 patients who developed hepatocellular carcinoma. These results indicate that chronic infection of hepatitis C virus may lead to hepatocellular carcinoma.

Key words: Hepatitis C virus (HCV) — Anti-HCV — Hepatocellular carcinoma — Chronic hepatitis

Hepatitis C virus (HCV) is now known to be the major cause of not only non-A, non-B acute hepatitis but also chronic liver diseases such as chronic hepatitis, cirrhosis of the liver and hepatocellular carcinoma.[1-3] There have been several reports describing patients with hepatocellular carcinoma developed after non-A, non-B acute hepatitis.[4-7] Recently, Tremolada *et al.*[8] described a case with non-A, non-B posttransfusion hepatitis who progressed to chronic hepatitis, cirrhosis and finally to hepatocellular carcinoma, and whose serial serum samples collected over 12 years were persistently positive for anti-HCV. To assess the role of HCV in the etiology of hepatocellular carcinoma, we followed patients with chronic hepatitis C for more than 7 years, and examined the evolution of anti-HCV and also compared the titer of anti-HCV between the stage of chronic hepatitis and that of cancer.

Fifty-eight patients with chronic hepatitis C were enrolled in this study. All patients were admitted to the Second Department of Internal Medicine, Shinshu University Hospital during the period from January 1970 to April 1990, and have been followed for 7 to 20 years with a mean of 13.2 years. They were 41 men and 17 women, with a mean age of 44.7 years. All patients had a history of blood transfusion and 20 of them were documented to have obvious clinical acute hepatitis. Initial diagnoses of chronic active hepatitis (26 patients) and chronic persistent hepatitis (28 cases) had been made on the basis of liver biopsy. The diagnosis of hepatocellular carcinoma was made on the basis of histological examinations, serological evaluation (α-fetoprotein) and examinations such as ultrasonography, computed tomography and selected hepatic angiography. The serum samples from all patients have been collected serially for up to 15 years, and stored at $-20°C$.

Anti-HCV was tested by means of an enzyme immunoassay using the HCV antibody ELISA test kit (Ortho Co., NJ). The anti-HCV titer was determined by dilution of serum samples with normal human serum and assay with a newly developed anti-HCV radioimmunoassay (RIA) test system employing a modification of Kuo *et al.*'s method[1] (Ortho Co., Tokyo and Ootsuka Laboratory, Tokushima). In the anti-HCV RIA test system, the beads were coated with recombinant HCV C100-3 protein and incubated with 20 μl of serum (diluted 1:11 with a buffer solution) for 2 h at 30°C. Bound antibody was detected by a second incubation with ^{125}I-labeled protein A. The radioactivity (cpm) of the processed beads was measured with a gamma counter. Using this system, representative serum samples gave a good linear association between cpm and dilution (fold) in the range from undiluted to 1:16. Standard serum containing anti-HCV which gave a cut-off index of 1.0 by Ortho ELISA test kit was tentatively defined as 1 unit. Titer was calculated by applying the following formula.

$$\text{Titer (unit)} = \frac{\text{cpm of tested sample} - \text{cpm of negative control}}{\text{cpm of standard sample showing 1 unit} - \text{cpm of negative control}}$$

The anti-HCV titer was tested in serum samples obtained at the phases of chronic hepatitis and of cancer in the 10

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Table I. Histological Outcomes of Chronic Hepatitis and the Evolution of Antibody to Hepatitis C Virus (Anti-HCV)

Final histologic diagnosis	No.	Transition of anti-HCV	
		+→+	+→−
Normal	4	0	4
Chronic hepatitis	30	30	0
Cirrhosis of liver	14	14	0
Hepatocellular carcinoma	10	10	0

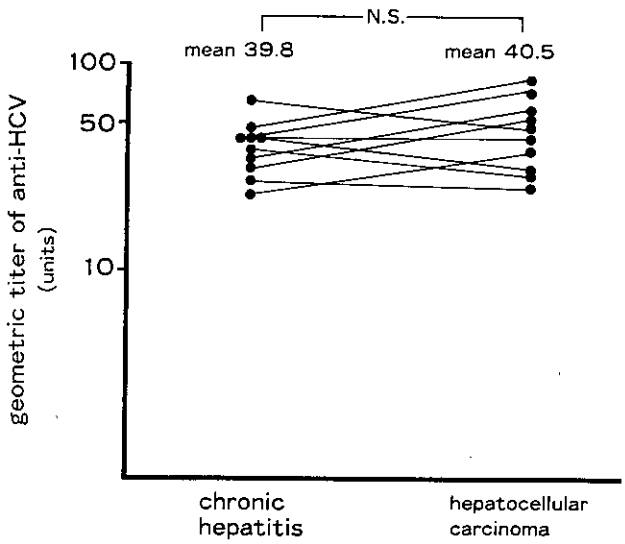

Fig. 1. Comparison of titers of anti-HCV at the stages of chronic hepatitis and of cancer in 10 patients who developed hepatocellular carcinoma. The interval between two serum samplings was 8.0±1.8 (mean±SD) years.

patients who developed hepatocellular carcinoma. The mean interval between the sampling times was 8.0±1.8 (mean±SD) years.

Table I shows the long-term outcomes of patients with non-A, non-B chronic hepatitis. Of 58 patients, 4 showed subsidence of disease biochemically and histologically, 14 developed cirrhosis of the liver, 10 developed hepatocellular carcinoma, and 30 remained as chronic hepatitis. Anti-HCV disappeared in the 4 patients whose hepatitis subsided, but it persisted in the remaining 54 patients. The mean titers of anti-HCV at the stages of chronic hepatitis and of cancer in the 10 patients who developed hepatocellular carcinoma were 39.8 units and 40.5 units, respectively, and the difference is not significant (Fig. 1).

A high prevalence of anti-HCV in patients with non-A, non-B hepatocellular carcinoma has been reported.[3,4] This does not necessarily imply a direct causal relationship between cancer and HCV, but represents circumstantial evidence. Our follow-up study in this paper revealed that anti-HCV persisted at a high level in all patients who showed continuous liver injury, including the patients developing hepatocellular carcinoma, and that it disappeared in patients whose liver disease subsided. These results indicate that anti-HCV reflects the continuing replication of HCV in the liver as suggested by Tremolada *et al.*[8] Furthermore, when the titer of anti-HCV was compared between the two stages of chronic hepatitis and cancer in the same patients who developed hepatocellular carcinoma, the mean titers at both stages were found to be at almost the same level. This result suggests that the replication of HCV in the liver may be similar throughout the clinical course. Kaneko *et al.*[9] succeeded in detecting the HCV-RNA in the sera of patients with type C hepatocellular carcinoma by the polymerase chain reaction method. So far, it is not known whether HCV virus is directly oncogenic or acts by inducing liver cirrhosis which leads to a mutagenic state associated with liver regeneration and nodule formation. Recently, Kew *et al.*[10] suggested the possibility of coexistence of HCV and hepatitis B virus in the pathogenesis of hepatocellular carcinoma in Southern African blacks. Indeed, we reported the high prevalence of coexistence of anti-HCV and hepatitis B surface antigen in Japanese patients with hepatocellular carcinoma.[11] However, the mechanism of carcinogenesis by HCV remains to be clarified in detail.

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