INTRANUCLEAR VIRUS-LIKE PARTICLES IN THE HEPATOCYTES OF THE PATIENTS WITH NON-A, NON-B HEPATITIS

Yukio GIBO, M.D., Atsuo NAGATA, M.D., Kendo KIYOSAWA, M.D. and Seiichi FURUTA, M.D.
The Second Department of Internal Medicine, Shinshu University School of Medicine, Matsumoto, Japan

Summary
Liver specimens were obtained from 21 non-A, non-B hepatitis patients when their serum transaminase levels were still elevated. The specimens were investigated by transmission electron microscopy in order to investigate the presence of the structures relating to this type of hepatitis. Intranuclear electron-dense aggregates of virus-like particles were observed in 2 out of 21 cases. One was an unresolved acute hepatitis case with short-incubation posttransfusion type, and the other was a chronic persistent hepatitis case without blood-transfusion. The size of these particles varied from 22 to 28 nm in diameter. The incidence of hepatocytes with the particles was approximately 2 to 3% of the cells investigated. These particles may represent an intranuclear alteration in close association with one type of non-A, non-B hepatitis.

Key Words: non-A, non-B hepatitis, intranuclear virus-like particles, transmission electron microscope.

Introduction
Two types of viral hepatitis, type A and type B have been diagnosed definitively by sensitive serological tests. It is well known that, after the exclusion of viral hepatitis of type A and type B as well as drug-induced liver injury, there remains a considerable number of patients with acute hepatitis either of the sporadic or post-transfusion variety, and this type is designated as non-A, non-B hepatitis.

Several papers have reported that human non-A, non-B hepatitis could be transmitted to chimpanzees1-4). This fact and other clinical aspects5-9) provide strong evidence that non-A, non-B hepatitis is caused by infectious agent(s). However, neither virus(es) of non-A, non-B hepatitis nor any serological markers associated with this type of hepatitis have been identified in a form universally agreed. Recently some peculiar tubular structures related with endoplasmic reticulum in the cytoplasm of liver cell10-12), or virus-like particles in the hepatocytic nuclei11,12) and liver homogenate13) of chimpanzees closely associated with non-A, non-B hepatitis have been observed by electron microscope. Virological studies of human non-A, non-B hepatitis, however, are still obscure, although virus-like particles have been observed in human serum or liver tissue14-18). This
paper reports the transmission electron microscopic study of the livers of non-A, non-B hepatitis patients especially focusing on the intranuclear virus-like particles observed in 2 cases, along with histological studies.

Patients and Methods

Twenty-one cases of non-A, non-B hepatitis were included in this study. Sixteen of them were with posttransfusion hepatitis (PTH) and the remaining 5 were sporadic cases. They were 7 males and 14 females, aged from 20 to 66 years (mean age 38.6). The diagnosis of non-A, non-B hepatitis was established according to the following serological tests; negative hepatitis B surface antigen (HBsAg) by reversed passive hemagglutination method, no response of antibodies to HBsAg by passive hemagglutination method, to hepatitis B core antigen by immune-adherence hemagglutination method, to hepatitis A virus by radioimmunoassay (HAVAB-M Kit, Abbott Laboratories, Chicago), to cytomegalovirus by complement fixation method, and to viral capsid antigen of Epstein-Barr virus by fluorescence antibody method between paired sera in the early and convalescent or late phases. The liver biopsy was performed, with the informed consent of the patients, during the phase of illness when their serum glutamic pyruvic transaminase (SGPT) levels were still elevated or fluctuated. The biopsied liver specimens were divided into 2 parts, one for light microscopy fixed in 10% formalin and processed routinely for hematoxylin-eosin and Azan-Mallory staining, and the other for the transmission electron microscopy (TEM). For TEM, the liver specimens were fixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide in phosphate buffer saline (pH 7.4), dehydrated through graduated concentration of ethanol and embedded in Epon 812. They were cut into ultrathin sections with LKB ultrotome using glass knife, and stained doubly with uranyl acetate and lead citrate. Then they were examined under Hitachi HU-11A electron microscope.

Results

The summarized data of clinical, histological aspects and the results of the TEM studies of these 21 cases are presented in Table 1. In 9 out of 21 cases, abnormal SGPT levels had continued for more than 1 year. Twelve cases revealed histological features of chronic hepatitis as reflected in portal or periportal fibrous expansion. The remaining 9 cases showed unresolved stages of acute viral hepatitis presenting unicellular and focal necrosis, sinusoidal lining cell activation and intralobular accumulation of neutrophile or mononuclear cells. On TEM investigation, 2 out of 21 cases showed virus-like particles in the hepatocytic nuclei as shown in Fig. 1 and 2. One is with PTH and the other with chronic persistent hepatitis without history of blood-transfusion. The clinical, histological aspects and the electron microscopic features of the intranuclear virus-like particles in these 2 cases were as follows:

Case 1: A 26 year-old nurse with frequent hypermenorrhagia followed by severe hypochromic anemia received a blood-transfusion of 1,600 ml. Fifty days later, she developed general fatigue and the peak activity of her SGPT levels was 389 Karmen unit (KU). Her subjective symptoms improved during admission, but her SGPT level fluctuated for 12 months of the follow up period. The diagnosis of non-A, non-B hepatitis was made and a liver biopsy was performed about 80 days after the onset of the disease. On light microscopy, her liver showed histological findings consistent with the diagnosis of unresolved acute viral hepatitis. On TEM, the aggregates of virus-like particles as shown in Fig. 1 were detected approximately 2% of the hepatocytic nuclei investigated. The size of these electron-dense par-