Seroconversion from HBs-Ag to Anti-HBs in a Case of Liver Cirrhosis Associated with Hepatocellular Carcinoma

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UNAKAMI, M., KOMIYA, I., ENDO, Y., HARA, M., IKEDA, K., KUMADA, H., YOSHIBA, A., HINO, O. and KITAGAWA, T. Seroconversion from HBs-Ag to Anti-HBs in a Case of Liver Cirrhosis Associated with Hepatocellular Carcinoma. Tohoku J. exp. Med., 1987, 152 (1), 81–86 — This paper reports a case of liver cirrhosis associated with hepatocellular carcinoma (HCC) of a woman who was converted from hepatitis B surface antigen (HBs-Ag) positive to antibody against HBs-Ag (anti-HBs) positive in the serum through an immunoregulatory steroid rebound phenomenon. The histology of the biopsy specimen taken before the seroconversion showed an early stage of liver cirrhosis with moderate infiltration of mononuclear cells. At autopsy about 3 years after the seroconversion, the liver tissue free of the tumor was in an early stage of liver cirrhosis. Fibrosis did not advance as compared with the biopsy specimen. In addition, mononuclear cell infiltration decreased remarkably and piecemeal necrosis disappeared after the seroconversion. The immunohistologic examination of hepatocytes demonstrated that positive stainings for HBs-Ag and for hepatitis B core antigen (HBc-Ag) in the biopsy specimen turned to be negative in the autopsy specimen. These facts indicate that the steroid rebound phenomenon eliminated free hepatitis B virus (HBV) in the hepatocytes in the absence of massive necrosis of hepatocytes. HBV-DNA integration was proved in the genome of HCC by molecular hybridization method. —— hepatitis B virus; seroconversion; steroid rebound phenomenon; HBV-DNA integration

This paper reports a case of liver cirrhosis associated with HCC. She was converted from HBs-Ag positive to anti-HBs positive in the serum through an immunoregulatory steroid rebound phenomenon caused by an interruption of steroid therapy. HBV-DNA integration was demonstrated in the genome of HCC by Southern blot-hybridization method.

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REPORT OF A CASE

A 62-year-old woman was admitted to the Toranomon Hospital with liver dysfunction. Biopsies in June 1976 and in June 1977 revealed an early stage of liver cirrhosis. HBs-Ag and hepatitis B e antigen (HBe-Ag) were positive in the serum. As her serum glutamic oxaloacetic transaminase (GOT) level elevated up to 600 KU in the course of following up at the outpatient clinic, she was readmitted. Prednisone was administered 30 mg per day for two weeks, and then it was gradually decreased by 5 mg. She was discharged with serum GOT at the level of 100 KU. The maintenance dose of corticosteroid was 10 mg every other day at this time. Soon after then, she discontinued to take steroid without consultation with any doctor. When she came to the outpatient clinic again, serum GOT was 400 KU. Thereafter, the level of GOT decreased gradually without medication. After this episode, HBe-Ag was converted to negative in April 1979 and antibody against HBe-Ag (anti-HBe) became positive in December 1979. HBs-Ag disappeared in January 1980 and anti-HBs appeared in the serum in March 1980. The level of serum GOT was within normal range. Repeated examinations by ultrasonography, scintigraphy and CT scanning had been unable to reveal any space occupying lesion in the liver until June 1983, when GOT elevated progressively. Multiple tumor nodes appeared and spread in the liver rapidly. The patient died in November 1983. The transition of serologic markers of HBV in the lapse of time is shown in Fig. 1.

PATHOLOGIC FINDINGS

Biopsy specimen. Portal tracts were infiltrated with mononuclear cells and their margins were irregular with piecemeal necrosis (Fig. 2). Fibrous septa often extended from enlarged portal tracts into lobules. Lobular architecture was distorted and pseudolobules were formed incompletely. HBs-Ag was observed scattered in hepatocytes immunohistologically (Fig. 3). HBc-Ag was positive in the nuclei of hepatocytes, and weakly positive in the cytoplasm (Fig. 4).

Autopsy findings. There was 1700 ml of thin bloody ascites. The liver was mildly enlarged (1800 g) and its surface was nodular. On the cut surface, the greater part of the liver was replaced by tumor nodes up to 2 cm in diameter (Fig. 5). A tumor embolus was stuck in the main trunk of portal vein. The tumor invaded into the hepatic vein and the inferior caval vein as well. Histologically

Fig. 1 Transition of serologic markers of HBV in the lapse of time. HBs-Ag, reversed passive hemoagglutination test; antiHBs, passive hemoagglutination test; HBeAg, cut off index; antiHBe, per cent (%) inhibition.
Fig. 2. Biopsy specimen. Portal tracts are enlarged with mononuclear cell infiltration and fibrosis. Piecemeal necrosis is noted. (Hematoxylin-eosin stain, ×25)

Fig. 3. The same biopsy as in Fig. 2. HBs-Ag is positive in the cytoplasm of hepatocytes. (Peroxidase-antiperoxidase method, ×125)

Fig. 4. The same biopsy as in Fig. 2. HBe-Ag is positive in the nuclei and in the cytoplasm of hepatocytes. (Peroxidase-antiperoxidase method, ×125)
the tumor was differentiated HCC, and tumor cells were arranged in a trabecular pattern. Tumor free liver tissue was in an early stage of cirrhosis. Mononuclear cell infiltration was very mild. There was neither piecemeal necrosis nor massive necrosis of hepatocytes (Fig. 6). Fibrous septa tended to be slender and occasionally consisted of only thin bundles of elastic fibers. Immunohistologically neither HBs-Ag nor HBc-Ag was detected in hepatocytes or in tumor cells. Carcinoma metastasis was noted in the bilateral lung and in para-aortic lymph nodes.

HBV-DNA integration. Tumor specimens were obtained from 4 different nodes of the tumor in the liver, and were investigated by Southern blot-hybridization method utilizing a $^{32}$P-labelled HBV-DNA probe which was made in Escherichia coli using plasmid pBR322 as a vector. The detail of the method
Seroconversion from HBs-Ag to Anti-HBs was published previously (Hino et al. 1984). As shown in Fig. 7, before DNA digestion with restriction enzymes, a smear of hybridization was seen in the high molecular weight region of the gel whereas after treatment with Hind III, two discrete bands were demonstrated. The integration pattern was identical in all of the 4 specimens. In order to exclude the possibility of plasmid DNA contamination, hybridization of cellular DNA of the tumor with pure plasmid pBR322 probe was performed and no discrete band was obtained. Unfortunately, HBV-DNA investigation of tumor-free portion of the liver was not done because of extensive invasion of the tumor in the liver.

**DISCUSSION**

The present case has shown that an interruption of steroid therapy in a patient with HBe-Ag positive chronic liver disease caused the seroconversion from HBe-Ag positive to anti-HBe positive with a temporary elevation of serum transaminase. Shortly after the HBeAg-antiHBe seroconversion, HBs-Ag disappeared and anti-HBs became positive in the serum. The immunohistologic examination of hepatocytes demonstrated that positive stainings for HBs-Ag and for HBe-Ag in the biopsy specimen turned to be negative in the autopsy specimen. This may suggest that hepatocytes with free HBV have been eliminated by the steroid rebound phenomenon. Moreover, it is worthy of note that mononuclear cell infiltration in the liver subsided remarkably and piecemeal necrosis disappeared after the seroconversion, and that the degree of distortion of lobular architec-
ture was almost the same in the biopsy and autopsy specimens. These facts indicate that the steroid rebound phenomenon suppressed the inflammatory process, and that it did not cause massive necrosis of hepatocytes.

As to the time of tumor occurrence, there are two possibilities. First, a min focus of carcinoma had been already present at the time of seroconversion. Second, after the seroconversion, the tumor arose from a hepatocyte with HBV-DNA integration. We consider that HCC occurred before seroconversion on the basis of our experience: We have 8 cases of HBsAg-antiHBs seroconversion caused by the steroid rebound phenomenon including the present case (Kumada et al. 1982). These cases have been followed up for 2 to 10 years, and HCC has occurred only in the present case. The steroid rebound phenomenon of other 7 cases occurred during the stage of chronic hepatitis and lead to the seroconversion. This fact suggests that the steroid rebound phenomenon which causes the seroconversion in an earlier stage of chronic hepatitis is effective to prevent the occurrence of HCC. The time of the steroid rebound phenomenon of the present case seems to be too late to prevent the development of HCC.

It is not known whether a hepatocyte with HBV-DNA is eliminated by the steroid rebound phenomenon. If a hepatocyte with HBV-DNA survives the steroid rebound phenomenon, a possibility cannot be denied that HCC occurred from a hepatocyte with HBV-DNA after the seroconversion.

The pattern of HBV-DNA integration was identical in the specimens obtained from 4 different nodes of the tumor in the liver. This indicates that the tumor arose from a single clone of hepatocytes despite the development of multiple tumor nodes in the liver.

Shafritz et al. (1981) reported that HBV-DNA had been integrated into the genome of hepatocellular carcinomas of patients who were seropositive for anti-HBs. However, little was known about past histories concerning serologic markers of HBV. The process similar to the present case is speculated to have been concerned in the HBV-DNA integration of HCC in some of their cases.

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