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Immunohistochemical Detection of Hepatitis B Core Antigen in the Bile Duct Epithelia

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SUMMARY : One hundred liver specimens of hepatitis B surface antigen positive cases were used for this study. The localization of hepatitis B core antigen in the bile duct epithelia was investigated by immunoperoxidase method. In three cases, hepatitis B core antigen was detected not only in the nuclei of the bile duct epithelia, but also in the cytoplasms of the bile duct epithelia. These findings suggested that hepatitis B virus may replicate in the bile duct epithelia. The resons for distribution of hepatitis B core antigen in the nuclei of the bile ducts are not known. A possible explanation may be that intrahepatic bile ducts develop from hepatocytes. To the best of our knowledge, morphologic localization of hepatitis B core antigen in the nuclei of the bile duct epithelia has not been reported previously.

INTRODUCTION

In 1965, Blumberg and collegues found an antibody in patients with viral hepatitis which react with an antigen in a single serum of Australian aborigine. Therefore, this antigen was called Australia antigen.[2] In 1977, Blumberg was awarded the Nobel prize for his discovery. Australia antigen is now known to be the surface of the hepatitis B virion and is termed hepatitis B surface antigen. Hepatocellular carcinoma becomes clinically manifest after cirrhosis has been well formed, and the major factor predisposing hepatocellular carcinoma in a population appears to be the presence of cirrhosis caused by chronic hepatitis B virus in the development of hepatocellular carcinoma.[20]

Hepatitis B virus infection is a significant public health problem in many countries. In Asian countries where infection with hepatitis B virus is hyperendemic, most people are infected during childhood through either mother-to-newborn ro child-to-child transmission. Antigenaemia develops in the baby within two months of birth and tends to persist. The peak incidence is in chilhood rather than in neonates. In such areas, including Africa, Greece, and Hong Kong intra-family spread seems particularly important. In the United States of America, hepatitis B virus infection is over 200,000 incidents of new infection of hepatitis B virus each year and 12,000 to 20,000 Americans annually join the ranks of chronic carriers.[1] The most effective procedure for controlling hepatitis B virus is the vaccination of newborn infants.[8,24] However, the current strategy of targeting high-risk groups for vaccination has not reduced the incidence of hepatitis B, and it has no chance of eradicating the disease in the United States of America.[10]

At Nagasaki in Japan, long-term trend of decreasing frequency of hepatitis B virus infection has been suggested.[21]

Histological location of hepatitis B core antigen in the bile duct epithelia has not been reported previously. Therefore, we investigated the morphologic localization of hepatitis B core antigen in extrahepatocyte areas. We present here the immunohistochemical findings of three autopsy cases with hepatitis B core antigen in the bile duct epithelia.

MATERIALS AND METHODS

The liver specimens from 100 autopsy cases associated with hepatitis B surface antigen positive at Nagasaki University Hospital were collected. These tissues were fixed in 10% formalin solution, dehydrated and embedded in paraffin. Sections were cut at four micron thickness for hematoxylin and eosin, Mallory's for collagen fibers, and silver impregnation for reticulum fibers stainnings. For immunohistochemistry, the sections were stained with anti-hepatitis B core antiserum (Dako PAP Kit) by using the peroxidase-anti-peroxidase (PAP) method.

The steps involved in the immunoperoxidase method for hapatitis B core antigen are as following: (1) Deparaffinize and hydrate in distilled water. (2) Treat with hydrogen peroxidase for 10 minutes in a 37°C incubator. (3) Wash in distilled water. (4) Wash in Tris buffer pH 7.4 using three cycle changes of 3 minutes each. (5) Treat with normal swine serum for 40 minutes in a 37°C incubator. (6) Tap off excess. (7) Treat with anti-hepatitis B core antibody (Dako) for 2 hours in a 37°C incubator. (8) Wash in Tris buffer pH 7.4 using three cycle changes of 5 minutes each. (9) Treat with swine anti-rabbit for 40 minutes in a 37°C incubator. (10) Wash in Tris buffer pH 7.4 using three cycle changes of 3 minutes each. (11) Treat with PAP for 40 minutes in 37°C incubator. (12) Wash in Tris buffer pH 7.4 using three cycle changes of 3 minutes each. (13) Treat with aminoethylcarbazole (AEC) as substrate solution for 40 minutes at room temperature. (14) Wash in distilled water. (15) Counterstain with Mayer's hematoxylin for 5 minutes. (16) Wash in distilled water. (17) Mount with glycerol gelatin (Sigma).

RESULTS

Immunohistochemical staining of hepatitis B core antigen resulted in red brown reaction products with AEC. In the bile duct epithelia three (3%) were hepatitis B core antigen positive out of 100 cases. Most of hepetitis B core antigen was localized in the nuclei of bile duct epithelia, sometimes it was localized in the cytoplasms (Figs. 1 and 2). Moreover, Immunoperoxidase investigation indicated that large number of hepatitis B core antigen was observed in the nuclei of hepatocytes and in the cytoplasms of hepatocytes (Fig. 3).

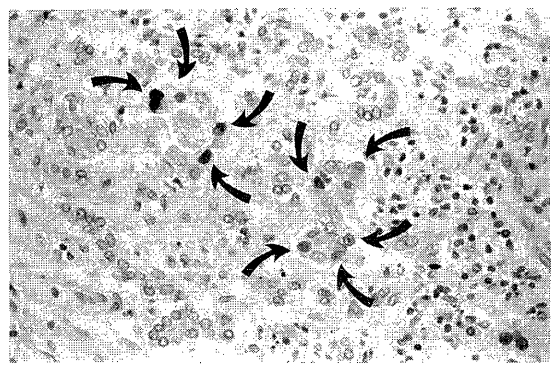

Fig. 1. Hepatitis B core antigen is localized in the nuclei of the bile duct epithelia (arrows). (Immunoperoxidase for hepatitis B core antigen, ×400).

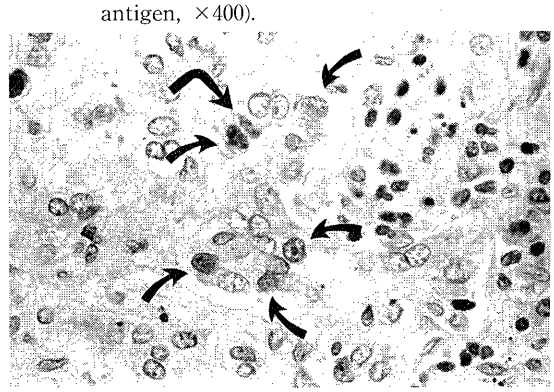

Fig. 2. Highter magnification of figure 1. Rarely hepatitis B core antigen is localized in the cytoplasm of the bile duct epithelium (square arrow), but most of hepatitis B core antigen is localized in the nuclei of the bile duct epithelia (arrows). (Immunoperoxidase for hepatitis B core antigen, ×400).

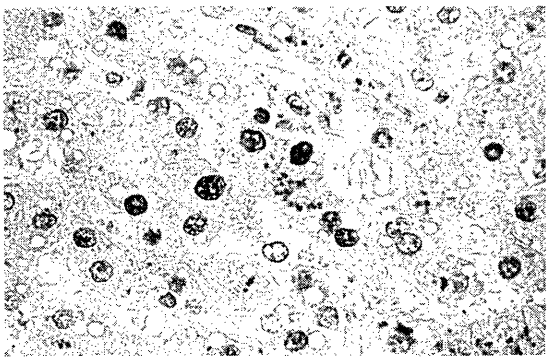

Fig. 3. Hepatitis B core antigen is observed in the nuclei of hepatocytes and also in the cytoplasms of hepatocytes. (Immunoperoxidase for hepatitis B core antigen, × 400).

DISCUSSION

The present study confirms the possibility of infection and replication of hepatitis B virus in the bile duct epithlia. It is suggested that hepatitis B virus has an affinity not only for the hepatocytes but also other cells. Based on light and electron microscopic studies, it has been suggested that intrahepatic bile duct cells develop from hepatocytes.[5] In contrast, other investigators have hypothesized that intrahepatic bile duct development occurs from ingrowth of epithelium of the extrahepatic bile ducts,[9] whereas some authors have proposed a combination of the two theories.[11] By cytokeratin immunohistochemistry, the development of the intrahepatic bile duct was studied in rat and human liver specimens.[4,6,7,22] It is concluded that the intrahepatic bile ducts develop from hepatocytes. Therefore, hepatitis B core antigen may replicate in the hepatocytes as well as in the bile duct epithelia.

The liver is thought to be the primary site of hepatitis B virus replication and synthesis. Hepatitis B virus antigen have been shown to be associated with both intrahepatic and extrahepatic diseases. Hepatitis B virus infection is association with the production of tissue damage outside the liver. Hepatitis B surface antigen and/or hepatitis B surface antigen immune complexes have shown in ex trahepatic localizations in renal glomeruli,[3,17] wall of vessels,[14] bone marrow and pancreas.[18,23,25] Localization of hepatitis B surface antigen in hepatocellular carcinoma has been reported.[13,15,16,19,26] Hepatitis B virus concentrated samples of saliva, urine and seminal fluid from HBeAg-positive male have shown the presence of HBV-DNA by molecular hybridization.[12]

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