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Detection of Hepatitis A Antigen in Human Liver

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For the first time, hepatitis A viral antigen (HAAg) was shown in liver biopsy tissue from a patient in the acute phase of hepatitis type A by light and electron microscopy, using the peroxidase-antibody technique. Under light microscopy, the staining for HAAg appeared as a fine, granular reaction product, scattered throughout the cytoplasm of hepatocytes and sinusoidal lining cells. Standard thin-section electron microscopy revealed virus-like particles, 24 to 27 nm in diameter, in cytoplasmic vesicles of hepatocytes and Kupffer cells. By immunoperoxidase electron microscopy, HAAg was detected on particles aggregated within cytoplasmic vesicles of hepatocytes, thus demonstrating that the virus-like particles (24 to 27 nm) are hepatitis A virus. The surrounding membrane of the vesicles was also positive for HAAg. The distribution patterns of HAAg in human liver were virtually identical to those described for experimentally infected marmosets. It is notable that most HAAg was detected within vesicles of liver cell cytoplasm, suggesting the possibility of vesicle-oriented morphogenesis of hepatitis A virus.

In a previous report (5), we demonstrated the light and electron microscopic distribution of hepatitis A viral antigen (HAAg) in liver tissue from marmosets experimentally infected with human hepatitis A virus (HAV), through the use of peroxidase-conjugated antibody. We also showed virus-like particles (24 to 27 nm) in vesicles located in hepatocytes and Kupffer cells. Similar HAAg particles have also been seen in cytoplasmic vesicles of hepatocytes from HAV-infected chimpanzees (6).

Thus far, however, attempts to detect HAAg in human liver have been unsuccessful, primarily because liver biopsy is infrequently indicated during the acute illness. In the present study, we describe the light and electron microscopic distribution of HAAg in liver tissue which was biopsied from a patient in the acute phase of the disease and labeled by the direct peroxidase-conjugated antibody technique.

MATERIALS AND METHODS

Patient. The patient was a 23-year-old female nurse on a hospital orthopedic service. On admission, she complained of general malaise, fever, aching, loss of appetite, and vomiting for 3 days. On physical examination, her temperature was 38.2°C, she was jaundiced, and it was noted that she had an enlarged, tender liver, without splenomegaly or lymph node swelling. Admission laboratory values revealed: serum glutamic oxalacetic transaminase (SGOT), 3,540 Karmen units (KU); serum glutamic pyruvic transaminase (SGPT), 5,076 KU; total/direct bilirubin, 6.3/3.2 mg/dl; alkaline phosphatase, 10.0 KA units (normal 4 to 10); prothrombin time, 88%; total cholesterol, 93 mg/dl (normal 180). There was no change in the patient's condition after 6 days in the hospital, but the laboratory values were SGOT, 270 KU; SGPT, 773 KU; total/direct bilirubin, 7.8/5.1 mg/dl; prothrombin time, 54%; and total cholesterol, 95 mg/dl. IgM anti-HAV, determined by solid-phase radioimmunoassay (HAVAB-M, Abbott Laboratories), was 4.7 (greater than 1.0 is a positive test). IgG anti-HAV was not determined. The increasing total bilirubin concentration combined with a decreasing transaminase concentration, decreasing prothrombin times, and a very low total cholesterol raised concern for the possible diagnosis of subacute hepatitis, an indication for liver biopsy. Percutaneous liver biopsy was performed on day 6 in the hospital, 9 days after clinical onset.

Liver tissue. Immediately after biopsy, the liver tissue was divided into three sections. One section was fixed with Formalin for routine histological examination, a second section with glutaraldehyde for standard thin-section electron microscopy, and a third section with periodate-lysine-paraformaldehyde for immunoperoxidase staining, according to McLean and Nakane (3). Liver tissues from patients with posttransfusion hepatitis type B or hepatitis non-A, non-B were prepared in the same manner and served as controls.

Preparation of peroxidase-conjugated antibody. Anti-HAV hyperimmune serum from chimpanzee no. 753 (2), with a titer of 16,000 by immune adherence hemagglutination assay, was used in the preparation of peroxidase-conjugates. $F(ab')_2$ fragments were prepared from purified immunoglobulin (Ig) G by pepsin digestion, treated with 2-mercaptoethanol, and conjugated with horseradish peroxidase as described by Nakane and Kawaoi (4).

Immunoperoxidase staining. We performed the direct conjugate staining method as follows. For light microscopy, liver tissue fixed with periodate-lysine-paraformaldehyde was sectioned into 4-μm preparates, incubated with peroxidase conjugates for 2 h, washed, fixed with 2.5% glutaraldehyde for 15 min, incubated in a 0.02% solution of 3,3′ diaminobenzidine (DAB) for 10 min in the presence of 0.002% hydrogen peroxide, lightly counterstained with hematoxylin, dehydrated, and mounted. For electron microscopy, tissue sections were incubated overnight with peroxidase-conjugate. After fixation with 2.5% glutaraldehyde for 20 min, sections were incubated in DAB solution for 30 min, followed by DAB solution containing 0.002% hydrogen peroxide for 10 min, postfixed with 1% osmium tetroxide for 30 min, dehydrated, and embedded in Epon 812 mixture. Epon blocks were examined under light microscopy for positively stained areas. Ultrathin sections were observed without additional staining.

RESULTS

Clinical course. Routine histological examination revealed a portal tract mononuclear cell infiltrate and periportal focal hepatocyte necrosis, findings consistent with acute viral hepatitis. The patient's condition improved, and she was discharged after 39 hospital days. Her laboratory values were SGOT, 50 KU; SGPT, 54 KU; total bilirubin, 1.5 mg/dl; prothrombin time, 91%; and total cholesterol, 159 mg/dl. IgM anti-HAV was less than 2.0 at 4 months after clinical onset, and IgG anti-HAV titer was 16,000 by immune adherence hemagglutination assay at 6 months.

Detection of HAAg by peroxidase-conjugated antibody technique. (i) Light microscopy. HAAg was readily detectable in the liver biopsy specimen from our patient (Fig. 1a). Finely granular peroxidase reaction products, indicating binding of antibodies to HAAg, were seen scattered throughout the cytoplasm of hepatocytes (arrows in Fig. 1a). Nuclei were negative for HAAg staining. Numerous sinusoidal lining cells were also positively stained. To verify the specificity of the peroxidase staining, the following controls were employed: (1) blocking tests, with paired unla-

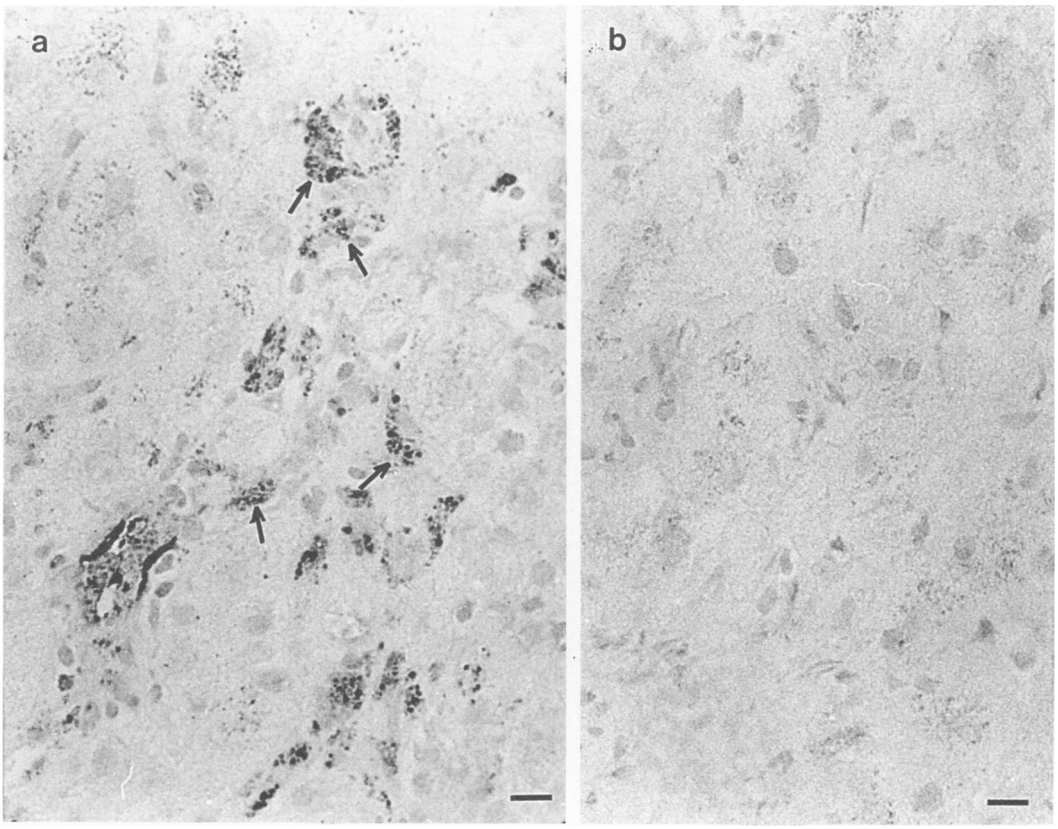

FIG. 1. Detection of HAAg by direct peroxidase antibody technique in liver biopsy tissue from a patient with serologically proven hepatitis type A. (Light microscopy, with hematoxylin counterstain; ×500; bar = 10 μm). (a) Finely granular reaction products (arrow) are scattered throughout the cytoplasm of hepatocytes; (b) control reaction: blocked peroxidase-labeled $F(ab')_2$ with HAAg by prior incubation with unlabeled anti-HAV serum.

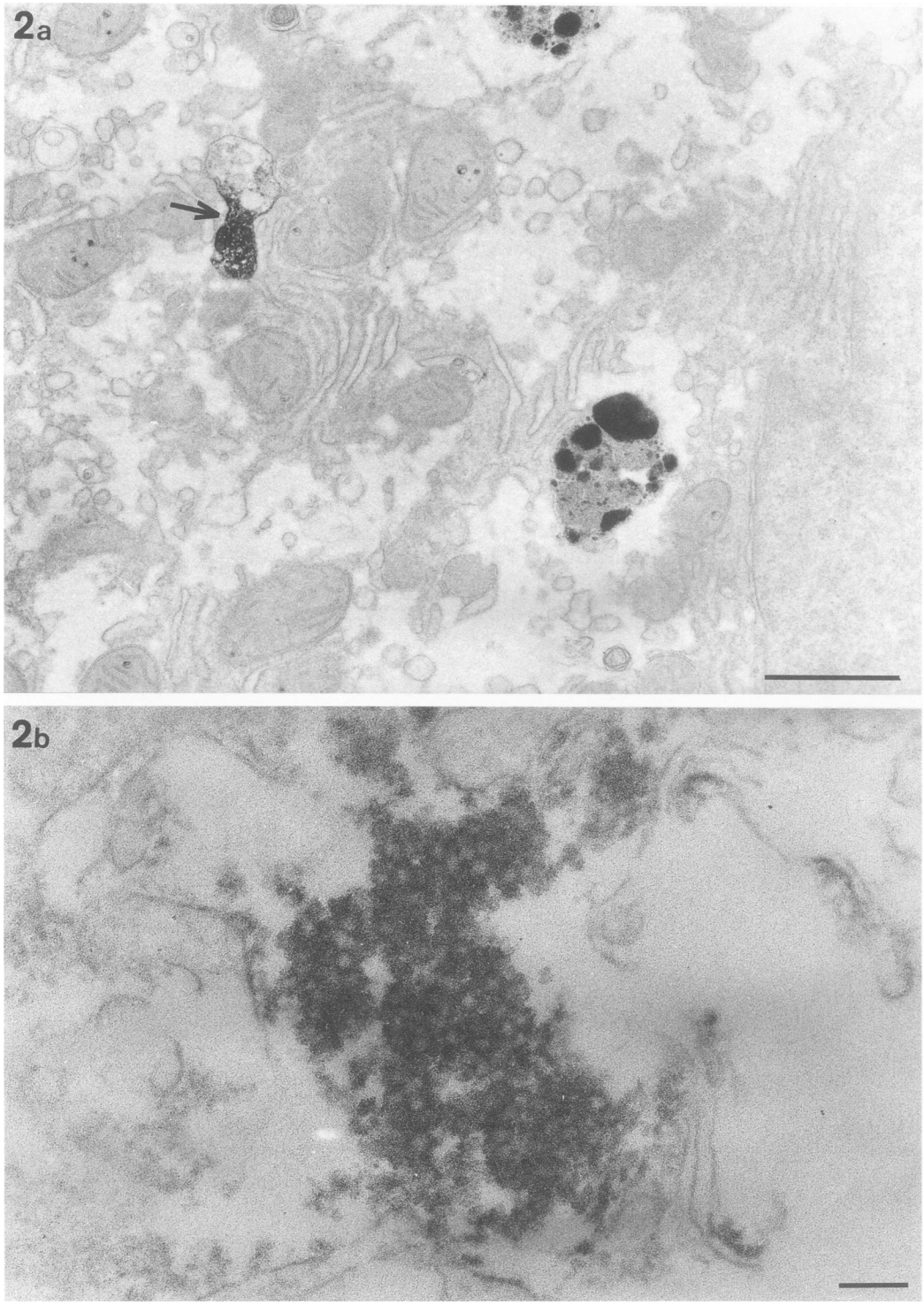

FIG. 2. Immunoperoxidase electron microscopy, demonstrating ultrastructural localization of HAAg by direct peroxidase antibody technique in a hepatocyte from a patient with hepatitis type A. (a) HAAg is located within a cytoplasmic vesicle (arrow). The surrounding membrane of the vesicle is also positively stained, (×15,000; bar = 1 μm); (b) higher magnification of the aggregate of the HAAg particles, (×100,000; bar = 100 nm).

beled preinfection and convalescent sera from a patient with serologically proven HAV infection; (2) omission of incubation with peroxidase conjugate to test for endogenous peroxidase activity in liver tissue; and (3) negative control liver biopsy tissues from patients with either posttransfusion hepatitis type B or non-A, non-B. Prior incubation with preinfection serum did not block peroxidase staining. In contrast, prior incubation with convalescent serum resulted in staining of scattered sinusoidal lining cells and minimal background staining (Fig. 1b). Tissue which was tested for endogenous peroxidase activity (not shown) demonstrated staining of scattered sinusoidal lining cells and minimal background staining, similar to tissue incubated with convalescent serum. There was no significant staining of liver tissue from negative control patients. Thus, the specificity of the peroxidase reaction was confirmed.

(ii) Electron microscopy. A positively stained area was selected for subsequent electron microscopic examination. HAAg was seen both in cytoplasmic vesicles of hepatocytes (Fig. 2a) and on the surrounding membranes of the vesicles (arrow in Fig. 2a). HAAg was not observed in nuclei. Under higher magnification (Fig. 2b), HAAg was seen on the particles aggregated within vesicles. The particles were coated with electron-dense reaction products and measured approximately 27 nm in diameter, without contribution to size by the reaction product. Similar HAAg particles were found in the cytoplasm of Kupffer cells.

Standard thin-section electron microscopy. Liver tissue examined by standard thin-section electron microscopy revealed virus-like particles in cytoplasmic vesicles of hepatocytes (arrow in Fig. 3). The particles measured approximately 24 to 27 nm in diameter. Both full and empty particles were seen. They appear predominantly in vesicles and frequently were accompanied by thin membranous structures. However, some

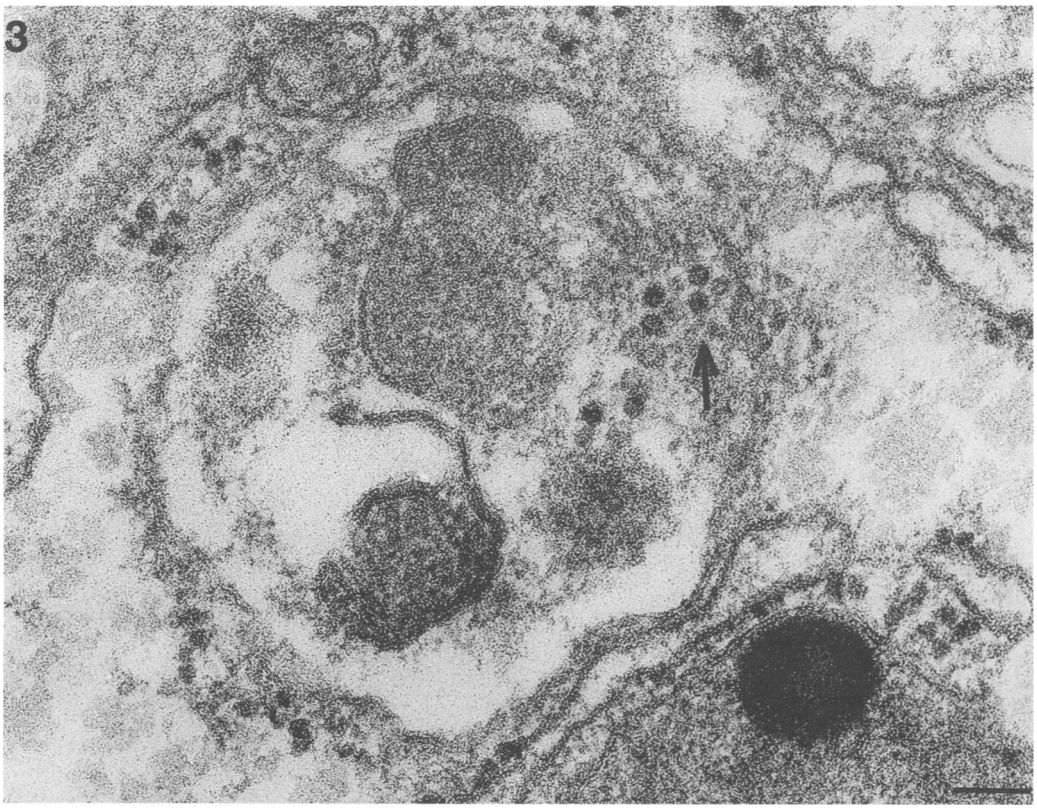

FIG. 3. Standard thin section electron microscopy, demonstrating virus-like particles (arrow) in a cytoplasmic vesicle of a hepatocyte from a patient with hepatitis type A, (×120,000; bar = 100 nm).

particles were seen in lysosomes. Similar particles (24 to 27 nm) were seen in Kupffer cell cytoplasm. No particles were observed in nuclei.

DISCUSSION

By utilizing techniques which have demonstrated HAAg in liver tissue from experimentally infected marmosets (5) and chimpanzees (6), we have successfully demonstrated HAAg-containing particles in hepatocytes and Kupffer cells from a patient with serologically proven hepatitis type A. The patient's presentation, clinical course, laboratory investigations, with the exception of the initial, markedly elevated transaminase concentrations and increasing total bilirubin concentration, and serology were typical of a sporadic outbreak of hepatitis type A without complications. This suggests that HAV can be readily detected in hepatic tissue within 1 to 2 weeks of clinical onset.

The ultrastructural distribution patterns we have described for HAAg in human liver were similar to those described for marmoset (5) and chimpanzee (6) liver tissue. In particular, it is notable that HAAg was found predominantly within cytoplasmic vesicles of all three species, whereas other small viruses, such as the enteroviruses, frequently appear as crystaline arrays in the cytoplasmic matrix of infected cultured cells.

Recently, Daemer et al. (1) reported the propagation of HAV in African green monkey kidney cell culture. A preliminary examination of HAV-infected African green monkey kidney cells has shown that HAAg and virus-like particles are located within cytoplasmic vesicles (unpublished data), suggesting the possibility of vesicle-oriented morphogenesis of HAV. Further examination will determine whether cytoplasmic vesicles are involved in the morphogenesis of HAV.

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