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Abstract

Liver biopsy specimens obtained from a 31-year-old female with delta-positive hepatitis were studied by routine electron microscopy. In several nuclei of hepatocytes, there were filamentous or microtubular structures 15 to 20 nm in diameter, in the vicinity of which, round particles, probably cross sections of tubular ones, were seen. In these nuclei, irregular granules approximately 20 to 30 nm in diameter were also found in clusters. However, cores of Dane particles were not found in such hepatocytes. These intranuclear microtubular structures may be associated with delta agent.

KEYWORDS: delta agent, delta antigen, intranuclear particles, microtubular structures

Tubular Structures in the Hepatic Nuclei of a Patient with Delta Agent

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Liver biopsy specimens obtained from a 31-year-old female with delta-positive hepatitis were studied by routine electron microscopy. In several nuclei of hepatocytes, there were filamentous or microtubular structures 15 to 20 nm in diameter, in the vicinity of which, round particles, probably cross sections of tubular ones, were seen. In these nuclei, irregular granules approximately 20 to 30 nm in diameter were also found in clusters. However, cores of Dane particles were not found in such hepatocytes. These intranuclear microtubular structures may be associated with delta agent.

Key words : delta agent, delta antigen, intranuclear particles, microtubular structures

The delta antigen which is identified in the liver and sera of hepatitis B virus (HBV) carriers, is an expression of infection with a new hepatitis agent whose interior contains an RNA genome and delta antigen (Ag) and whose exterior is coated by hepatitis B surface antigen (HBsAg)(1). Originally described in Italy, delta infection seems to be world-wide, but in Japan there is very little evidence of delta infection, despite the prevalence of HBsAg carriers (2). Recently, we have found a patient with intrahepatic delta Ag and sero-antibody to delta Ag (anti-delta).

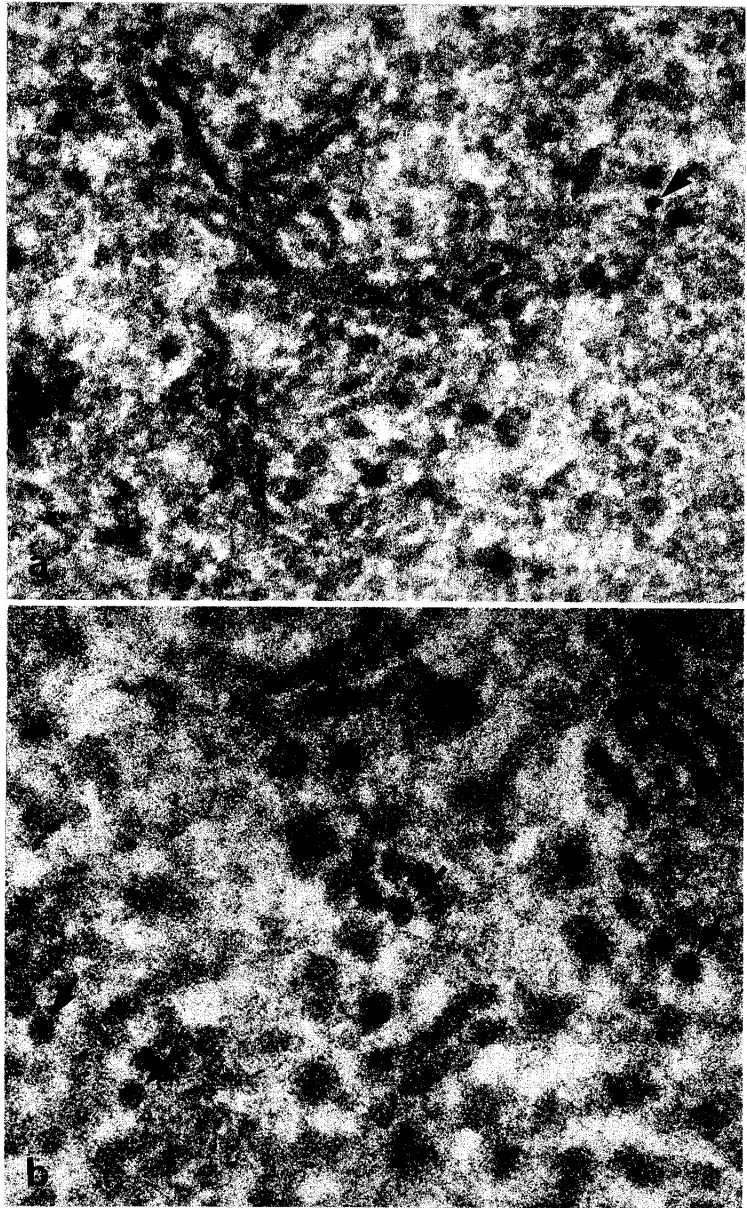
In previous studies, no specific ultrastructural markers of delta infection have been found in the liver (3). In this study, we demonstrated intranuclear filamentous or microtubular structures in hepatocytes of a patient with delta agent by routine electron microscopy.

Liver biopsy specimens were obtained from a 31-year-old female who had HBsAg-positive chronic hepatitis and in whose

serum a high titered plateau of anti-delta was detected by radioimmunoassay (Abbott Laboratories, North Chicago, Illinois). The specimens were divided into three portions. One portion fixed with Bouin's solution was used for conventional light microscopy. Another portion fixed with periodate-lysine-paraformaldehyde (4) was stained with Fab' of anti-delta IgG conjugated with horseradish peroxidase (HRPO) according to Nakane and Kawaoi (5), HRPO-labeled antibody to HBsAg (6) and HRPO-labeled antibody to hepatitis B core antigen (HBcAg)(7). The remaining portion was fixed with glutaraldehyde and osmium tetroxide, and embedded in Epon. The ultrathin sections were stained with uranyl acetate and lead citrate and examined under a Hitachi 700-H electron microscope.

Under the light microscope, using the peroxidase-labeled antibody method, delta Ag was observed in scattered hepatic nuclei, and HBsAg was found in the cytoplasm of some hepatocytes. HBcAg was absolutely

Fig. 1 a, Intracellular filamentous or microtubular structures in a hepatocyte of a patient with delta agent. In their vicinity, small round particles (arrows) are also observed. $\times 120,000$. b, Higher magnification of small round particles (arrows). Limiting membrane and core are discernible. $\times 240,000$.



negative in hepatocytes.

By routine electron microscopy, cores of Dane particles were not found in nuclei of hepatocytes. In several nuclei of hepatocytes, however, filamentous or microtubular structures 15 to 20 nm in diameter were found in clusters of three or more (Fig. 1). In their vicinity, there were small round

particles, approximately 15 nm in diameter, which consisted of a limiting membrane and an electron opaque core (Fig. 1). They seemed to be cross sections of tubular forms. Another irregular type of particles from 20 to 30 nm in diameter were also observed in clusters (Fig. 2a). They were morphologically different from the cores of

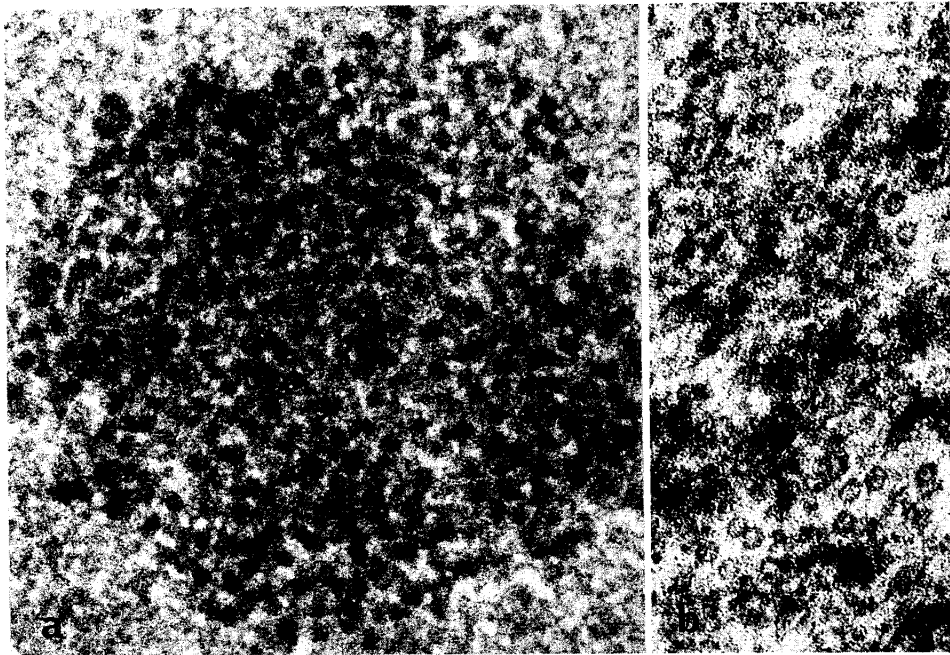


Fig. 2 a, A cluster of irregular intranuclear granules in a hepatocyte of the same patient as in Fig. 1. $\times 120,000$. b, Intranuclear cores of Dane particles in a different patient with chronic type B hepatitis. $\times 120,000$.

Dane particles detected in a different patient with chronic hepatitis B (Fig. 2b)

Studies in chimpanzees inoculated with delta-positive hepatitis have shown that the delta agent may represent a transmissible, defective virus which requires HBV as a helper (8). In isopycnic and rate zonal sedimentation analysis, it is reported that delta Ag co-purifies with a 35 to 37-nm subpopulation of HBsAg particles in sera (1). On the other hand, in the liver, delta Ag localizes mainly in nuclei and occasionally in cytoplasm of hepatocytes (9). However, no virus-like ultrastructures specific to delta Ag were found in the liver of experimental animals or patients by routine or immunoelectron microscopy (2, 8). Intranuclear granules 20 to 30 nm in diameter were occasionally reported. Similar granules were demonstrated in our patient, but they have also been observed in hepatocytes of hepatitis B or non-A, non-B (NANB)

hepatitis patients (10, 11). In this paper, we showed clusters of filamentous or tubular structures in nuclei of some hepatocytes. Similar filamentous or microtubular forms were not found in hepatic nuclei of other biopsy specimens obtained from patients with chronic hepatitis B or NANB (10). Furthermore, the size of intranuclear tubular forms did not correspond to intracisternal HBsAg-positive tubular structures approximately 25 to 30 nm in diameter (6). The relation of intranuclear tubular structures to granules is being investigated. Additional routine and immunoelectron microscopic studies are essential to determine the mode of formation of intranuclear tubular forms and to further characterize them.

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