NOTES

Ultrastructural Studies of Hepatitis A Virus by Electron Microscopy

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Well-defined, core-like structures were visualized in hepatitis A virus particles by a modified microelectron microscopy technique.

In 1969, Almeida and Waterson reported the development of immune electron microscopy (IEM) for the visualization of low-titer virus particles (1). IEM has been employed extensively by our laboratory (4) and others (3, 6) in studies involving the isolation and biophysical characterization of the hepatitis A virus (HAV). However, the morphological characteristics of HAV have been difficult to assess by IEM, since identifying antibody normally obscures the features of the virus particle. We now report the results of a modified microelectron microscopy technique for the study of HAV ultrastructure.

A marmoset, no. MB-90 (*Saguinus mystax*), was inoculated with acute illness-phase plasma obtained from patients in the 1973 common-source outbreak of hepatitis A in Phoenix, Arizona (4, 7). The marmoset was sacrificed shortly after onset of elevated serum isocitric acid dehydrogenase activity; its liver was removed and processed (5) for subsequent biophysical and electron microscopic studies. A small portion of this liver preparation was used in an attempt to develop a modified micro-IEM test for hepatitis A.

The usual IEM preparation in our laboratory has contained 0.2 to 0.8 ml of antigen. It seemed reasonable that a micromethod, wherein 0.02 to 0.05 ml of antigen were used, would conserve precious HAV antigen, thus enabling us to perform additional IEM tests. Briefly, the micro-IEM test involved the addition of 0.02 ml of a 1:20-diluted antibody source (convalescent human serum) and 0.05 ml of the marmoset MB-90 liver preparation in a microtiter "V" plate (Cooke Engineering Co., Alexandria, Va.). This was then incubated for 1 h at room temperature and then for 2 h at 4°C. A second preparation was made as a control by mixing

0.02 ml of MB-90 liver preparation and 0.02 ml of triple distilled water in another V plate (microelectron microscopy [EM] method) just before grid preparation. Both samples were then prepared for EM viewing by placing 0.02 ml of each sample on a parlodion/carbon-coated 300-mesh copper grid. The sample was allowed to adsorb to the grid for 1 to 2 min. The excess solution was blotted off with filter paper. The grids were then stained with 2% phosphotungstic acid (pH 7.0, without bovine serum albumin). Grids were examined with an RCA EMU-4 or a Philips 201C at a screen magnification of about 45,000×.

Although the micro-IEM method was not found to be an acceptable procedure for visualizing HAV particles, the companion EM procedure yielded extremely interesting findings. Using the micro-EM method, we were able to visualize particles with a morphology characteristic of HAV. In addition, these particles appeared to contain a clearly defined, internal core-like structure (Fig. 1) not previously discernible by the IEM method. In addition to the well-defined core-like structure seen in several of the virus particles, there also appeared to be a double-layered coat around the core. One possible interpretation is that the inner coat is actually a nucleocapsid structure, whereas the outer coat is comprised of protein capsomers. If this is, in fact, the case for HAV, then it would appear to be different from the majority of enteroviruses that lack a defined core and are characterized by a single protein shell and a ribonucleoprotein core (2). It should be emphasized that this is a presumptive comparison of HAV with the enterovirus class of viruses, since the nucleic acid type of HAV has not been definitely established.

There is little doubt the virus particles we

FIG. 1. *Hepatitis A particles by micro-EM with well-defined internal structure (original magnification, 187,000 $\times$).*

FIG. 2. Hepatitis A particles from same liver preparation as Fig. 1, but internal structure obscured by antibody used for IEM (original magnification, 187,000 $\times$).

have seen by direct micro-EM are HAV, since IEM experimentation of the MB-90 liver preparation confirmed the presence of numerous antibody-coated HAV particles (Fig. 2). Moreover, no other particles not coated by antibody were seen in the IEM preparation. In addition, many HAV particles viewed by the usual IEM method also appeared to contain core-like structures. However, the cores were not as clearly defined and appeared to vary in size; the antibody coating obviously degraded the EM image of the particle. The spherical particles found by modified micro-EM in our study also conformed to the 25- to 30-nm size accepted for HAV virus (4).

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