Non-A, Non-B Epidemic Hepatitis: Visualization of Virus-like Particles in the Stool by Immune Electron Microscopy

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SUMMARY

Acute-phase stool samples collected from hepatitis cases during outbreaks of water-borne epidemic hepatitis were examined by immune electron microscopy (IEM). Spherical virus-like particles (27nm in diameter) were visualized in the stool of a hepatitis patient with serological evidence of non-A, non-B (NANB) hepatitis. The IEM demonstrated serological specificity of the antigen with the patient's own convalescent serum as well as a known pool of NANB hepatitis convalescent sera. It is suggested that these virus-like particles may be the aetiological agent of faeco-orally transmitted NANB epidemic hepatitis in India.

Until recently, water-borne epidemics of hepatitis in India were believed to be due to the faeco-orally transmitted hepatitis A virus (HAV). Recent research has shown that the causative agent(s) of the disease is neither HAV nor hepatitis B virus (HBV) but a postulated new entity termed non-A, non-B (NANB) hepatitis (Khuroo, 1980; Wong et al., 1980). The identification of this form of hepatitis is mainly based on exclusion of HAV and HBV infection and clinico-epidemiological manifestation of the disease.

Balayan et al. (1983) reported the isolation and visualization of virus-like particles from the stool of a volunteer after oral administration of a pooled faecal extract from NANB hepatitis patients. Since the sero-epidemiological studies on water-borne epidemic hepatitis in India indicated a possible excretion of the aetiological agent in the faeces, we examined the stool samples of hepatitis cases by immune electron microscopy (IEM). We report here the detection of 27-nm diameter, spherical virus-like particles in the stool of a patient with serological evidence of NANB hepatitis.

During the course of an investigation of a water-borne epidemic of hepatitis at Kolhapur, Maharashtra State, India, between February and March 1981, 22 stool samples were obtained from patients 2 to 5 days post-onset of illness. Ten stool samples were collected from patients with hepatitis in Pune between September and October 1982. Among the Pune stool samples, five were collected during a focal outbreak while the remaining five were obtained from sporadic cases. Five to 10% suspensions of each stool in phosphate-buffered saline were examined by the techniques of IEM described previously (Kapikian et al., 1972) with minor modifications. The source of antibody for IEM was: (i) convalescent serum of the patients from whom stool samples were collected; (ii) convalescent sera (pooled) obtained from five patients during an epidemic of NANB hepatitis at Kolhapur; (iii) known anti-HAV serum from chimpanzees (kindly supplied by Dr R. H. Purcell, NIH, Bethesda, Md., U.S.A.).

To determine whether the virus-like particles detected in the stool sample belonged to any of the common enterovirus groups, the stool suspension was inoculated into primary monkey (Macaca radiata) kidney cells, the buffalo green monkey (BGM) cell line and 1-day-old infant mice. The inoculated cell cultures were observed for 10 days for c.p.e. The mice inoculated intra-cerebrally and subcutaneously were observed for sickness for 15 days and two blind passages of brain suspension were carried out.
Serological studies had not demonstrated HBV antigenaemia in any of the acute or convalescent phase sera by enzyme-linked immunosorbent assay. Only one of the 22 patients from Kolhapur and three of the 10 from Pune had specific antibody of IgM class to HAV. The stool samples of these HAV-positive patients were devoid of virus-like particles by IEM. Of the remaining 21 stool samples from Kolhapur, coronavirus-like particles were seen in two cases and spherical 27- to 29-nm virus-like particles in one case but without any evidence of immune aggregation. Thus, these virus-like particles could not be incriminated as aetiological agents of the disease. Of the ten Pune stool samples, one displayed immune aggregation of spherical virus-like particles after incubation with the patient's own convalescent serum and the pooled NANB convalescent sera (Fig. 1 a, b). Immune aggregation was not observed in the same stool sample when reacted with the known anti-HAV chimpanzee serum.

It is now generally accepted that human faeco-orally transmitted NANB hepatitis is a separate entity distinct from post-transfusion NANB hepatitis (Dienstag et al., 1981). The visualization and characterization of this agent is of primary importance for the development of serological techniques for diagnosis of the disease.

In the IEM studies, we have shown immune aggregation of virus-like particles in the stool of a natural case suffering from NANB hepatitis. The immune aggregates were observed after incubation of the stool suspension with the patient's own convalescent serum as well as pooled convalescent sera collected during a known NANB hepatitis epidemic at Kolhapur (Fig. 1 a, b). It is of interest to note that the pooled convalescent sera obtained from Kolhapur contained antibodies against the NANB hepatitis agent of Balayan et al. (1983) by IEM (M. S. Balayan, personal communication). Fairly large aggregates (10 to 28) of virus-like particles were seen when the stool suspension was incubated with a 1:10 dilution of the patient's serum as well as pooled convalescent sera. In contrast, the stool suspension incubated with the higher dilutions of convalescent sera demonstrated smaller aggregates (3 to 6). The immune aggregation of morphologically similar virus-like particles in the Pune stool sample seems specific since these particles were coated with antibody derived from the patient's own convalescent serum. The pooled convalescent serum also showed immune aggregates of similar virus-like particles. The stool sample did not show immune aggregation with a known chimpanzee-derived anti-HAV serum. This suggests that these virus-like particles may not be closely related to HAV. Anti-HAV IgM was also not detected in the serum of this case obtained 90 days post-onset of illness. Moreover, the virus-like particle appeared to be non-pathogenic for infant mice, and c.p.e. was not observed in primary monkey kidney and BGM cells. This suggested that the virus-like particles detected by IEM may not represent a common enterovirus present in the stool. The
spherical virus-like particles had an average diameter of 27 nm with a range between 26 and 29 nm and morphologically resembled picornaviruses.

The above observation of IEM coupled with the serological studies suggests that the 27-nm virus-like particles visualized in the stool of NANB hepatitis patient may be the aetiological agent of this disease.

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REFERENCES


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