Morphological Irregularities in Dane Particle Cores

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Summary

Electron microscopy of hepatitis B antigen has revealed Dane particles with abnormal morphology. These aberrant Dane particles contain incomplete cores. They were seen in large numbers in the serum of an HBsAg-carrying renal dialysis patient and were less numerous but invariably present in other Dane particle-containing sera — 12 from asymptomatic carriers, 2 chronic hepatitis patients and 1 patient with acute hepatitis. All 16 sera were shown to contain HBeAg.

It is generally accepted that the Dane particles, found in hepatitis B antigen-positive sera, represent the hepatitis B virus and that the smaller spherical and filamentous components consist of excess virus coat material.

In most instances, the Dane particles are roughly spherical and 42 nm in diam. They contain an antigenically distinct inner core of approx. 27 nm in diam. The outer coat of the Dane particles is usually positioned close to the core surface in concentric fashion, and is occasionally extended to form a filamentous appendage giving the particle the appearance of a tadpole (Fig. 1 a).

Cores may be released from Dane particles by detergent treatment. These (Fig. 1 b) often display hexagonal outlines and give the appearance of having a regularly arranged surface structure, which suggests that they possess icosahedral symmetry and, as such, probably represent the virus capsid. Similar observations were made by Budkowska (1977) on core particles isolated from the nuclei of hepatocytes.

A standard procedure was used to screen samples of serum for the presence of hepatitis B surface antigen (HBsAg) by electron microscopy. Samples of 0.5 ml of serum were diluted 1/10 with 0.05 M-phosphate buffer pH 7.2 (PB). These were clarified by centrifugation at 10,000 g for 10 min. The supernatant fluids were centrifuged at 45,000 g for 1 h and the pellets were resuspended in PB and subjected to two further high speed centrifugations. Final pellets were resuspended in a drop of distilled water, mixed with an equal vol. of 2% phosphotungstic acid, pH 6.1 and examined in a Siemens Elmiskop 1 A.

The serum of a renal dialysis patient was examined at intervals over a period of 5 years. This patient was an HBsAg carrier and his serum consistently contained HBsAg of conventional morphological appearance. A moderate number of Dane particles was invariably present. After four and a half years, routine screening of his serum revealed a striking difference in the proportion of the various HBsAg components — there was a marked increase in the number of Dane particles and decrease in the number of spheres and tubules, so that the ratio of Dane particles to smaller components was in the order of 1:2. Moreover, most of the Dane particles were smaller than usual, whereas others were much larger, and their shapes did not conform with the usual spherical appearance (Fig. 1 c).

Closer examination revealed that these irregular Dane particles contained cores which were incomplete or structurally abnormal. Fig. 2(a to f) illustrates some of the morphological variations which were observed. Dane particles were frequently seen to be bean-shaped (Fig. 2 a). The structure of the cores within these particles was always defective and resembled icosahedral shells in varying stages of completion. This is particularly evident in one particle illustrated (Fig. 2 a, asterisk) where the coat has partially stripped away from the core. The core structures varied from less than a hemisphere to almost complete capsids.
Fig. 1. (a) Dane particles 42 nm in diam. showing conventional morphology. Two ‘tadpoles’ are present. (b) Cores released from Dane particles by detergent treatment. Many show hexagonal outlines and regular surface structure suggesting icosahedral symmetry. (c) HBAg from the serum of a renal dialysis patient shows a high proportion of abnormal Dane particles.

with possibly just a few missing capsomers. Sometimes a small dense spot or ring could be seen in the position comparable to the hilum of a bean. The outer coat of these aberrant Dane particles followed the contours of the defective core so that the final shape of the Dane particle depended upon the ‘completeness’ of the core. Occasionally, an invagination of the outer coat extended into the centre of the core through its incomplete shell (Fig. 2b). This
Fig. 2. (a) Bean-shaped Dane particles containing incomplete cores. Where the outer coat has partially stripped away (asterisk) the deficiency in the core structure is particularly evident. (b) Malformed Dane particles in which the outer coat has formed an invagination into the centre of the incomplete core. (c) Particles in (b) as seen from above. (d) ‘Tadpole’ containing a defective core. (e) Central dense spot or ring-shape in spherical Dane particles might represent different views of these same structures seen at the ‘hilum’ of the bean-shaped particles (shown in a). (f) Particle containing an apparently complete core measuring only 22 nm in diam. (g, h) Two defective cores, within a single coat. (i) Dane particles unpenetrated by stain show surface ‘dimples’. The large particle with two dimples may contain two defective cores. (j, k) Cores released from Dane particles in Fig. 1 (c) are clumped by patient’s own anti-HBc. Incomplete cores (arrows), as well as 22 and 27 nm cores, are present in the complexes.
form, positioned so that the indentation was centrally placed on the upper surface of the particle, appeared in the electron microscope as a spherical particle with a central ‘dimple’ filled with stain (Fig. 2c). Bean-shaped particles similarly positioned (i.e. with electrons passing through the ‘hilum’) may well give rise to variations illustrated in Fig. 2e, where Dane particles with apparently ‘normal’ shape are seen to contain a central ring-shape or dense spot.

Defective cores were also seen in the tadpole forms (Fig. 2d). It may be significant that in these cases the tail of the tadpole was always seen to emerge from the side of the particle where the capsid was incomplete or irregular.

A number of Dane particles contained cores which appeared to be complete structures but which were much smaller than usual, measuring only 20 to 22 nm in diam. (Fig. 2f). Similar smaller-than-average core particles have been observed (Stannard et al. 1973a) in the post mortem liver homogenate of an HBsAg-positive patient.

A most unusual and rare observation was the finding of two cores within a single coat (Fig. 2g and h). In each case, both core particles were smaller than average and both defective in structure. The large particle, illustrated in Fig. 2(i), which has not been penetrated by stain, has two ‘dimples’ and might therefore conceivably contain two defective cores.

It is not illogical to presume that the Dane particles with structurally defective cores might also be lacking in DNA. Insufficient serum was available for DNA polymerase activity determinations. There have been numerous reports of a strong correlation between the presence of e antigen (HBeAg) and DNA polymerase activity (Nordenfelt & Andrén-Sandberg, 1976; Cappel et al. 1977; Tong et al. 1977). The serum with a high percentage of defective Dane particles was found to be strongly positive for HBeAg by immuno-diffusion (ID) tests in gel. This finding on its own does not necessarily imply the presence of DNA polymerase. HBeAg in serum has been associated with the presence of numerous Dane particles (Takahashi et al. 1976) which in turn have been shown at times to contain a circular DNA molecule and endogenous DNA polymerase (Robinson et al. 1974; Robinson & Greenman, 1974). It follows therefore that both HBeAg and DNA polymerase will frequently be detected in the same Dane particle rich serum samples, but their simultaneous occurrence need not be inevitable. It is possible that HBeAg may be a product completely unrelated to the hepatitis B DNA polymerase, but connected instead to the production of structural proteins of Dane particles — coats or cores.

Immune electron microscopy was used to determine whether the defective core particles were antigenically similar to the cores with conventional morphology. Preparations of HBsAg from the serum were treated to remove the coats of the Dane particles and expose the cores to the anti-HBc present in the patient’s serum. This was accomplished by first concentrating the HBsAg (together with some of the immunoglobulins) by centrifugation at 60,000 g for 2 h. The pellet was resuspended in PB containing 0.25% sodium deoxycholate (w/v) and 0.6 units/ml protease (from Streptomyces griseus) and incubated at 37 °C for 2 h. Previous experience (Moodie et al. 1974) had shown that the Dane particle coats could be completely removed by this protease digestion in the presence of the bile salt. The treated preparation was prepared for electron microscopy by repeated cycles of centrifugation.

Results showed immune complexes of cores containing complete particles 27 nm and 22 nm in diam., as well as numerous defective core particles which had incomplete shells (Fig. 2j, k). From this observation it was possible to conclude that the malformed core particles were antigenically similar (although not necessarily identical) to the cores which were morphologically intact.

It is not possible to tell from these studies whether the cores were incomplete because of defects in the assembly process, or whether they were complete cores which had become
damaged before acquiring their outer coats. Although it has been clearly shown that cores are assembled within the nuclei of hepatocytes (Huang, 1971), the site at which the outer coat is added has not yet been demonstrated. The fact that the outer coat of the Dane particle consistently follows very closely the contours of the core, suggests that the outer coat is not obtained by a budding process.

The discovery of these aberrant Dane particles in the serum of a renal dialysis patient led to a re-examination of other HBsAg-positive sera which had been studied by electron microscopy. A recent investigation of 150 serum samples from blood donors who were asymptomatic carriers of HBsAg showed that Dane particles could be detected in 36% of samples, and in large numbers in 12 samples (8%). These figures are consistent with previous observations (Stannard et al. 1973b). The 12 sera with numerous Dane particles invariably contained some particles which exhibited similar morphological variations. These irregular Dane particles were not usually numerous and had therefore frequently been overlooked. They were also detected in the serum of one patient with acute hepatitis and sera from two patients with chronic hepatitis. HBeAg was detected by ID in all 15 sera except one, from an asymptomatic carrier, which was shown to be positive for HBeAg by RIA.

The production of defective Dane particles appears to occur with varying frequency in different individuals, but their presence could not be attributed to any one clinical state associated with HBsAg antigenaemia. The reason for the high percentage of such particles in the serum of the renal dialysis patient must remain one of speculation. As the patient has since died, further investigations will unfortunately not be possible.

Similar Dane particle irregularities have been shown by other workers. Desmyter et al. (1972) reported on the morphology of Dane particles and referred to ‘coiled’ structures which, at that time, they could not easily reconcile with known virus morphology. From their illustrations it is obvious that the ‘coiled’ structures were in fact similar to the unusual Dane particles described in the present report. It is also possible that some of the ‘crescentic forms’ of HBsAg described in an early report by Almeida (1972) may well be malformed Dane particles.

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REFERENCES


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