Dane Complexes in Hepatitis B Antigen

(Accepted 22 April 1974)

SUMMARY

Complexes consisting of only Dane particles are found frequently in Hepatitis B antigen-positive sera. It is proposed that the reason for their presence is the existence of a third antigen-antibody system involving the Dane coat material but not the small spheres or tubules.

It is commonly accepted that the 42 nm double-coated Dane particle of the Hepatitis B antigen (HB Ag) is the most likely candidate for the Hepatitis B virus particle and that the 20 to 25 nm tubules and the 20 to 22 nm small spheres are excess Dane coat material in various stages of ageing. Structures identical to the tubules have been observed frequently to be continuous with the outer coat of the Dane particles resulting in the formation of ‘tadpole’ forms, and the tubules in turn appear to break up into the small spherical components.

Electron microscopy of antigen-antibody complexes has shown that the coats of the Dane particle share an antigen or antigens with the tubules and the small spheres since each component can be bound to the others by addition of antibody specific to surface antigens (anti-HB).

It has been demonstrated clearly by Almeida, Rubenstein & Stott (1971) that the inner 27 nm cores of the Dane particles are antigenically distinct from their coats. This is consistent with a nucleocapsid–envelope relationship in which the envelope consists predominantly of host cell material and therefore is antigenically distinct. The cores have the further nucleocapsid properties of production without envelopes in the nuclei of the hepatocytes (Huang, 1971) and a morphology consistent with that of an icosahedron. They appear to have a surface structure of regularly repeating subunits (Fig. 1, arrows) which frequently suggests four subunits along the edge of one facet (Fig. 1, inset). Stannard et al. (1973a) have shown also that the IgG and IgM molecules of the core antibody (anti-HB0) from a donor serum, attach to the core particle surface at different and specific sites, in a manner similar to that observed by Brown & Smale (1970) with another icosahedral virus, that of foot-and-mouth disease.

Many enveloped viruses are known to have a virus-coded antigen incorporated in their host-cell derived envelopes and this may be true for the coats of Dane particles. The following observations have led us to believe that the Dane coats contain an antigen or antigens not demonstrable in the small spheres or tubules and that a third antigen-antibody system may be added to the two previously described (Almeida et al. 1971; Stannard et al. 1973a).

Aggregates consisting almost entirely of Dane particles are common in the examination of Hepatitis B positive sera by electron microscopy (Field & Cossart, 1971; Zalan et al. 1971; Stannard et al. 1973b). We have also found a number of sera which contain large quantities of Hepatitis B antigen, including numerous Dane particles, but with no detectable antibodies to surface antigens. In the electron microscopy of these sera, aggregates were rare and these invariably contained Dane particles alone or a mixture of Dane particles and ‘tadpoles’ only (Fig. 1). Attached antibody molecules could not be identified with certainty although thin irregular strands appeared between the particles.

These same sera examined by electron microscopy after agitation in chloroform for
Fig. 1. Complex of Dane particles and 'tadpoles' from untreated serum. Indistinct strands are visible between particles. Arrows indicate cores showing regular surface structure. Inset: group of cores with subunits visible at their peripheries.

Fig. 2. A large complex of Dane particles and 'tadpoles' formed after exposure of the serum to chloroform.
Fig. 3. Complex formed after deoxycholate treatment contains damaged Dane particles, coats and cores. Anti-HB, molecules are visible only on the exposed cores.

Fig. 4. Complex of cores alone formed after treatment first with protease and then with deoxycholate.
Short communications

5 s, or treatment with approx. 0.01% mucosal overnight at room temperature, showed many and larger complexes of Dane particles including ‘tadpole’ forms (Fig. 2). The smaller spherical components remained unaggregated but were damaged, the tubules had become more obviously beaded and were broken into smaller pieces, and both tubules and small spheres were more easily penetrated by negative stain. The action on the lipoprotein of either chloroform or detergent altered the surface structure of the Dane coats and the small spheres and tubules, but since the latter remained unaggregated, the complexing of the Dane particles was considered to be specific and significant.

The Hepatitis B antigen was concentrated with the immunoglobulins by differential precipitation of these sera containing Hepatitis B antigen with polyethylene glycol of mol. wt. 6000. After treatment of this antigen-immunoglobulin concentrate overnight with 1% of the detergent, mucosal or with 0.25% of the bile salt, deoxycholate, the tubules and small spheres showed the same morphological damage while the Dane particles were characteristically ruptured to expose the inner cores. These damaged Dane particles, free cores and free coats were aggregated into large complexes (Fig. 3). The core antibody molecules, invariably present in these sera free of surface antibody, were attached to the exposed cores and linked some cores together. However, many core-less coats were present in the complexes and some of the core particles were apparently entirely surrounded by partially damaged coats which prevented core-to-core attachment by their specific antibody. It is therefore unlikely that the coats were mechanically trapped within a complex of cores, especially since the complexes were repeatedly washed and centrifuged before electron microscopy.

It is possible that exposure of the Dane particles to detergent or chloroform initially altered the coats in such a way as to expose antigenic sites which were usually obscured and, as a result, the first complexes were formed with the damaged Dane particles. After extended detergent treatment the coats were further disrupted to allow the cores to combine with their specific antibody. To explain the presence of the few Dane particle complexes found in untreated serum samples, it would be necessary to postulate that some coat damaging process had occurred which allowed the additional antigenic sites to become exposed.

Additional evidence for a cryptic antigen in the Dane coats is provided by the effects on Hepatitis B antigen of protease followed by deoxycholate. When protease (0.6 unit/ml) alone was added to the antigen-immunoglobulin concentrate and incubated for 1 h at 37 °C, this had very little effect upon the morphology of the antigen. However, when the protease treatment at 37 °C was followed by deoxycholate treatment at 4 °C overnight, the electron micrographs were very different from those obtained by exposure of the antigen to the bile salt alone. Small spheres and tubules were present in damaged form but not aggregated. There were no Dane particles and no Dane particle coats, but there were large complexes of Dane particle cores linked together by anti-HBc, immunoglobulin (Fig. 4) in a manner similar to that shown by Stannard et al. (1973a) for the IgG of anti-HBc on the core surface.

These observations suggest the presence of a hidden Dane-coat antigen which is not present in the 20 nm small spheres or in the tubules and which is destroyed by this protease treatment. It may account for the frequent appearance in HB Ag positive sera of complexes of Dane particles only.
The authors wish to thank Dr E. R. Rudman, Western Province Blood Transfusion Service, South Africa, for the plasma containing Hepatitis B antigen.

M.R.C./U.C.T. Virus Research Unit
Medical School
Observatory, Cape
South Africa

REFERENCES


(Received 26 February 1974)