Hepatitis B Antigen: IgG Components Shown by Immune Electron Microscopy

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SUMMARY

Immune complexes could be formed with hepatitis B Ag and anti-IgG serum after treatment of the antigen with detergent, CsCl or glycerol, but not before. It is suggested that an antigenic determinant specifically reacting with anti-IgG forms an integral part of hepatitis B Ag. This determinant is ordinarily obscured in the undamaged antigen.

The morphological appearance of hepatitis B antigen (HBAg) has been well documented and it has been established that the surface antigen (HBsAg) is antigenically distinct from that of the inner core of the Dane particles (HBoAg). Two antigen–antibody systems with unrelated specificity have been demonstrated (Almeida, Rubenstein & Stott, 1971; Stannard et al. 1973). In addition, it has been suggested (Moodie, Stannard & Kipps, 1974) that the coats of the Dane particle contain a hidden antigen specific for the 42 nm particles and not contained in the spheres and tubules.

Recently there has been considerable discussion about the possibility of host serum components being incorporated in HBsAg. Millman et al. (1971) showed that purified HBsAg after treatment with 1% Tween 80 was able to produce precipitin lines with antisera to several human serum components suggesting that the latter were therefore an integral part of the HBsAg. Neurath, Prince & Lippin (1974) employed affinity chromatography on columns of insolubilized antibodies to plasma proteins and demonstrated that purified HBsAg adsorbed to columns containing antibodies to a variety of serum components including IgG, chain. As treatment of the HBsAg with detergents or proteolytic enzymes failed to prevent this adsorption, they concluded that the antigenic determinants related to human plasma proteins were constituent components of HBsAg. Using purified [3H]-labelled HBsAg, Burrell (1975) showed that HBsAg particles have tightly bound antigenic determinants which react at low affinity with antisera to certain normal human serum components, but there it was uncertain whether these were integrated into the structure of HBsAg particles.

Employing the technique of immune electron microscopy, it was possible to visualize the reactions between HBsAg and anti-human IgG globulin. Hepatitis B Ag was obtained from a carrier whose serum had been shown to be negative for HB,Ab by CEP but positive for HB,Ab. Electron microscopy showed the HBsAg to be uncomplexed and randomly distributed (Fig. 1a). Occasional clusters of presumably damaged Dane particles as previously reported by Moodie et al. (1974) were seen.

The HBsAg from the serum was concentrated by centrifuging at 60000 g for 60 min and resuspending in phosphate buffer, pH 7.2 (PB). Samples of this antigen suspension were allowed to stand overnight in each of the following: (a) PB (control), (b) 2% Mucosal detergent at room temperature, (c) 15% CsCl at 4°C, (d) 25% glycerol at 4°C. Thereafter
Fig. 1. (a) Untreated HBAg is seen to be randomly scattered and remains uncomplexed after the addition of anti-IgG. (b) After treatment with 2% Mucasol the HBAg appears damaged but unaggregated. (c) Mucasol-treated HBAg becomes complexed by the addition of anti-IgG. (d) HBAg exposed to 15% CsCl is shown to form complexes with anti-IgG.
the HBAg was removed from the suspending medium and washed by centrifuging, re-
suspended in PB and incubated in the presence of goat anti-human IgG at 36 °C for 1 h.
Samples were washed twice before examination in the electron microscope by negative
staining using 1 % PTA, pH 6.0.

Detergent treated HBAg loses some of its morphological integrity (Fig. 1 b). The Dane
coats tend to rupture. The tubules and spheres become distorted allowing negative stain to
penetrate but, as in the untreated control sample, they remain uncomplexed and freely
scattered. Addition of anti-IgG globulin to HBAg after detergent treatment results in the
formation of large numbers of complexes (Fig. 1 c) containing spheres, tubules and Dane
particles. Identical results were obtained using anti-IgG, anti-IgG (Fc) or anti-IgG (Fab).
Untreated HBAg, on the other hand, remained unaggregated in the presence of anti-IgG,
suggesting that the antigenic determinant involved in complex formation with anti-IgG is
not on the surface of the HBAg particles, but can be exposed by the action of detergent.
This observation is consistent with the findings of Millman et al. (1971). However, contrary
to their results with Tween 80, it was demonstrated by immune electron microscopy that
large immune complexes could be formed with Mucasol treated HBcAg and specific anti-
HB,Ab.

Treatment with either CsCl or glycerol resulted in no detectable morphological change of
the HBAg, but in each case the treated antigen was found to be complexed by the addition
of anti-IgG (Fig. 1 d), although to a lesser extent than after treatment with detergent. Un-
treated control HBAg remained consistently unaltered in the presence of anti-IgG.

Neurath et al. (1974) were able to demonstrate the presence of an antigenic determinant
reacting with anti-IgG as an integral component of HBAg without prior detergent treatment
and found that subsequent detergent treatment did not alter their results. It is of interest that
part of the procedure used by this group for the purification of HBAg, involved its exposure
to 10 to 50 % glycerol in tris buffer for 15 h.

Burrell (1975) employed discontinuous sucrose gradients containing 14.3 % CsCl for the
concentration of their HBAg prior to testing for reactivity with antisera to normal serum
components. He reported that 28 % CsCl alone resulted in considerable antigen losses. It is
our experience that exposure of HBAg to 15 % CsCl for 16 h results in alteration or damage
sufficient to allow complex formation with anti-IgG.

The observations made by immune electron microscopy favour the conclusion that HBAg
contains an integral antigenic determinant which combines specifically with anti-IgG. This
determinant is not normally exposed on the surface of the particles but can be uncovered by
the action of certain agents including detergents, CsCl and glycerol. Whilst it is essential
to remove all possible contaminating serum components from the HBAg before studying its
basic chemical construction, care should be exercised to ensure that preliminary purification
procedures do not create architectural rearrangement or displacement.

It is interesting to speculate whether the potentially damaging procedures (such as banding
in CsCl, use of proteolytic enzymes, etc.) usually employed in the purification of HBAg for
the production of a specific antiserum in animals, may account for some of the difficulties in
raising a monospecific antiserum uncontaminated by antibodies to normal serum proteins
(Boenisch & Katz, 1971).

It remains to be ascertained whether the antigenic determinants able to combine with
anti-IgG consist of either conventional serum globulin or whether they are in fact non-
related cross-reacting antigens.

Immune electron microscopy may prove to be a useful tool in determining the specificity
of serological reactions between HBAg and antisera to other normal serum components as it
Short communications

is a method by which immune complexes can readily be distinguished from non-specific aggregates.

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REFERENCES


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