Association of Hepatitis B e-Antigen (HBeAg) Determinants with the Core of Dane Particles

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SUMMARY

Immunoprecipitates obtained by reacting Dane particle cores with human antibodies to hepatitis B core antigen (HBcAg) were chromatographed on columns of Sepharose 4B CL using 3 M-NaSCN as eluant. An antigen having the size and immunological specificity of monomeric e-antigen (HBeAg) was separated from HBcAg by this method. Antisera from animals immunized with HBcAg or HBeAg reacted not only with the antigen used for immunization but also with HBeAg and HBcAg, respectively. This indicates that HBeAg determinants are associated with the core of Dane particles.

The detection of hepatitis B e-antigen (HBeAg) in sera is correlated with the presence of Dane particles (Nordenfelt & Kjellén, 1975; Takahashi et al. 1976), with infectivity of the sera (Alter et al. 1976; Okada et al. 1976; Beasley et al. 1977; Shikata et al. 1977) and with the detection of hepatitis B core antigen (HBcAg) in liver nuclei (Murphy et al. 1976; Trepo et al. 1976). Although the antigenic determinants of HBcAg and HBeAg can be distinguished on the basis of reactions with human antibodies (anti-HBc and anti-HBe, respectively (Takahashi et al. 1976; Neurath et al. 1978c)) the following preliminary findings suggest that HBeAg determinants may be associated with the nucleoprotein core of Dane particles: (a) a Rhesus monkey antiserum to HBcAg, purified from hepatitis B virus (HBV)-infected livers (antiserum V805-501-563 supplied by the Research Resources Branch of the National Institute of Allergy and Infectious Diseases, Bethesda, Md.), was positive for anti-HBe (J. Vnek, personal communication) in a radioimmunoassay (RIA) test (Mushahwar et al. 1978); (b) apparently monomeric HBeAg is similar to HBcAg with respect to isoelectric point and contains a polypeptide having the mol. wt. of the major component of HBcAg (Neurath et al. 1978a, c); (c) purified HBcAg (Neurath et al. 1978a) after reduction and alkylation, carried out as described (Neurath et al. 1978c), followed by treatment with ethanol (final concentration 50 to 60 %, v/v; Budkowska, 1977) lost its reactivity with anti-HBc in agreement with results described by Budkowska (1977) but was positive for HBeAg as determined by the respective RIA tests (Neurath et al. 1978b, c). Results presented here provide further evidence for the physical association of HBeAg determinants with HBcAg.

Although human anti-HBc IgG isolated from sera containing HBeAg reacted in RIA tests only with HBcAg but not with HBeAg (Neurath et al. 1978c), the Rhesus monkey antiserum mentioned before contained both anti-HBc and antibodies to the apparently free and monomeric HBeAg described earlier (Neurath et al. 1978c; Table 1). The presence of anti-HBe in the antiserum could be due either to the presence of HBeAg-determinants on HBcAg used for immunization or due to contamination of the HBcAg preparation with HBeAg. To distinguish between these two possibilities, rabbit antisera to HBeAg were prepared and their reaction with monomeric HBeAg and with HBcAg was followed by RIA tests. Human anti-HBe isolated from sera of hepatitis B surface antigen (HBsAg)
Table 1. Anti-HBc and anti-HBe antibodies in human HBsAg carriers and in animals immunized with Dane particle cores or with HBeAg

<table>
<thead>
<tr>
<th>Characterization of serum</th>
<th>Anti-HBc titre*</th>
<th>Anti-HBe titre†</th>
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<tbody>
<tr>
<td>Human anti-HBc</td>
<td>1:5000</td>
<td></td>
</tr>
<tr>
<td>Human anti-HBc (from HBeAg-positive serum)</td>
<td>1:5000</td>
<td>&lt; 1:2</td>
</tr>
<tr>
<td>Rhesus monkey anti-HBc‡</td>
<td>1:5000</td>
<td>1:320</td>
</tr>
<tr>
<td>Rabbit anti-HBe§</td>
<td>1:16</td>
<td>1:128</td>
</tr>
<tr>
<td>Rabbit anti-HBc¶</td>
<td>1:12</td>
<td>1:24</td>
</tr>
<tr>
<td>Rabbit anti-HBc¶</td>
<td>1:800</td>
<td>1:16</td>
</tr>
</tbody>
</table>

* Corresponding to 50% inhibition end-points (Neurath et al. 1978b). Dane particle cores used in the test = 10 μl of fraction No. 14 (Fig. 1).
† Corresponding to 50% inhibition end-points. Apparently monomeric HBeAg (Neurath et al. 1978c) was used in the test. The relationship between anti-HBc or anti-HBe titres and the absolute concentration of the respective antibodies is not known.
‡ Immunizing antigen = Dane particle cores isolated from infected livers.
§ Prepared as described by Neurath et al. (1976). The antigen used for immunization consisted predominantly of HBeAg bound to IgG.
¶ The antigen used for immunization was apparently monomeric HBeAg free of HBcAg and HBsAg determinants as determined by RIA tests.
†† The antigen used for immunization was HBcAg corresponding to fractions 14 + 15 (Fig. 1).

carriers could not be used for this purpose since these sera also always contain antibodies to HBcAg determinants. Results shown in Table 1 indicate that the rabbit anti-HBe sera were positive in an RIA inhibition test for anti-HBc. This suggests that HBeAg determinants are exposed on a considerable portion of HBcAg particles.

More direct evidence for the physical association of HBeAg determinants with HBcAg is based on the following experiment. Dane particles were sedimented from about 300 ml of HBeAg-positive serum (which had been clarified by centrifugation at 10,000 rev/min for 20 min) at 25,000 rev/min for 5 h in the SW 25-2 rotor (Beckman Instruments, Palo Alto, California). The pellet was resuspended in 10 ml of ‘disruption buffer’ (0.1 M-NH4Cl−0.08 M-MgCl2−0.25% mercaptoethanol−0.05% Nonidet P40 (NP40), pH 7.6; Neurath et al. 1978) and incubated for 30 min at 37 °C in order to release HBcAg from Dane particles. The preparation was dialysed overnight against 0.01 M-tris (hydroxymethyl) aminomethane−0.14 M-NaCl−0.02% NaN3 (TS) containing 0.1% NP40. Anti-HBe IgG (2.5 ml; titre determined by RIA = 1:1,000; Neurath et al. 1978b) isolated from HBcAg-positive serum and free of anti-HBe detectable by RIA was added to precipitate HBcAg. The mixture was incubated for 30 min at 37 °C, overnight at 4 °C, layered on top of 10 ml of 15%, v/v, glycerol and centrifuged at 25,000 rev/min for 1 h in the SW 25-1 rotor. The pellet was suspended in 2 ml of TS, layered on top of 2 ml of 15% glycerol and centrifuged at 25,000 rev/min in the SW 65 rotor for 1 h. The final pellet was dissolved in 3 M-NaSCN and chromatographed by gel filtration on a column (1.5 × 30 cm) of Sepharose 4B CL (Pharmacia, Piscataway, New Jersey) as described in Fig. 1. Fractions after gel filtration and the supernatant from the last centrifugation were analysed by RIA for HBcAg, HBeAg and HBsAg. The relative concentrations of each of these antigens were determined from RIA calibration curves. To distinguish HBeAg from HBcAg in RIA tests, duplicate samples, diluted with either normal human IgG or with anti-HBc IgG (isolated from HBeAg-positive serum), were tested. Similar ct/min indicated the presence of HBeAg, while suppression of ct/min to background levels by anti-HBc indicated the presence of HBcAg (Neurath et al. 1978c). The first peak of radioactivity representing the RIA-positive material
Fig. 1. Gel filtration on a column of Sepharose 4B CL of an HBcAg-anti-HBc immune complex. Arrows correspond to the column void volume (1), to the peak positions of HBsAg [2; mol. wt. 2.5 to 3.7 x 10^6 (Howard & Burrell, 1976)], apoferritin (3; mol. wt. 4.5 x 10^6), and to the included volume of the column (4); 3 M-NaSCN was used as eluant and one ml fractions were collected. Influenza virus and phenol red were used as markers for the void and included volumes of the column, respectively. Samples (50 µl) of fractions dialysed against TS containing 3 µg/ml of phenylmethylsulphonylfluoride were tested by RIA, measuring both HBeAg and HBcAg (Neurath et al. 1978c). Radioactivity corresponding to negative controls (25 ct/min) was subtracted from the experimental results.

eluted just behind the void volume of the column corresponds to HBcAg for the following reasons: (a) its elution pattern is consistent with a mol. wt. of about 9 x 10^6 established for Dane particle cores by gel filtration (Fields et al. 1977); (b) addition of 100 µl of human anti-HBc to fractions corresponding to this peak resulted in negative RIA tests; (c) positive RIA results were obtained when beads coated with anti-HBc IgG (isolated from HBeAg-positive sera) instead of IgG containing both anti-HBc and anti-HBe (Neurath et al. 1978c) were used; (d) only trace amounts of HBsAg were detected in the fractions, indicating nearly complete removal of the Dane particle envelope from the nucleoprotein core. The second peak of radioactivity corresponds to HBeAg since: (a) re-chromatography of pooled fractions 31 to 37 (concentrated to 1 ml) on Sephadex G-100 using 3 M-NaSCN as eluant revealed that the RIA-positive material had a mol. wt. of approx. 35000, established earlier for apparently monomeric HBeAg (Neurath et al. 1978c); (b) addition of human anti-HBc to fractions corresponding to this peak failed to cause any inhibition in RIA tests but addition of human IgG containing both anti-HBc and anti-HBe resulted in negative RIA tests; (c) RIA tests were negative with beads coated with anti-HBc only; (d) addition of rabbit anti-HBe to the fractions resulted in negative RIA tests.

The concentration of HBeAg in the re-dissolved immunoprecipitate used for chromatography was about 50 times higher than in the last supernatant after sedimenting the HBcAg–anti-HBc complex, while the level of HBsAg in the supernatant was approx. 10 times higher than in the combined fractions after gel-filtration. Therefore, HBeAg recovered from the HBcAg–anti-HBc immunoprecipitate cannot represent a contaminant from ‘soluble’ HBeAg present in the original serum.
The co-precipitation of HBeAg determinants with the nucleoprotein core of Dane particles by anti-HBc (free of anti-HBe) and the finding that HBcAg exposed to 3 M-NaSCN reacted with animal anti-HBe and elicited an immune response to HBeAg (Table 1) suggests that HBeAg is a component of Dane particle cores released from the cores under appropriate conditions. It remains unknown whether HBcAg and HBeAg determinants are located on distinct proteins, or whether they represent antigenic determinants on the same protein in two distinct conformations as suggested (Neurath et al. 1978 c).

Lam et al. (1977) concluded that HBeAg was released from Dane particle-rich preparations treated with detergents. However, their experiments failed to distinguish between HBcAg and HBeAg determinants. The interaction of Dane particles with anti-HBe observed by us (Neurath et al. 1976) but not confirmed by others (Gerin et al. 1978; Takahashi et al. 1978) may be at least partly ascribed to exposure of HBeAg determinants on partially damaged Dane particles (J. Vnek, personal communication) in analogy with exposed HBcAg determinants on such particles (Purcell et al. 1974).

An association between HBeAg and HBcAg seems to be supported by the localization of these antigens in nuclei of HBV-infected livers (Arnold et al. 1977). However, antigenic sites reacting with antibodies directed against both HbcAg and HBeAg were detected at the membrane of infected hepatocytes in cases of acute lobular hepatitis and chronic active hepatitis (Huang & Neurath, 1978), suggesting that internal HBV components may be localized on membranes of infected cells in analogy with other viruses (Tung et al. 1976; Biddison et al. 1977; Braciale, 1977; Ledbetter et al. 1977; Virelizier et al. 1977). If indeed HBeAg may become exposed on the surface of HBV-infected hepatocytes, an immune response to HBeAg may play an active role in modulating the production of Dane particle cores and in eliminating cells involved in their biosynthesis. Such mechanisms would offer a reasonable explanation for the negative correlation between the presence of anti-HBe in serum and the synthesis of HBcAg and HBV.

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REFERENCES


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