Release of e Antigen from a Dane Particle-Rich Preparation of Hepatitis B Virus

KUI CHUN LAM, MYRON J. TONG,* AND JORGE RAKELA
Liver Unit, University of Southern California School of Medicine,* and the Rancho Los Amigos Hospital, Downey, California 90242

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Tween 80 treatment of a Dane particle-rich pellet obtained from sera of a carrier with a hepatitis type B infection resulted in the release of e antigen.

The e antigen (HBeAg), recently discovered by Magnius and Espmark (6), has attracted considerable interest because of its possible use as an indicator of viral infectivity as well as for its ability to predict the clinical outcome in patients infected with hepatitis B virus (HBV; 7, 10). HBeAg is found only in type B hepatitis infections and is distinct from both the hepatitis B surface antigen (HBsAg) and the hepatitis core antigen (6, 12). The fact that it is most commonly detected in the presence of circulating Dane particles and HBV-specific deoxyribonucleic acid (DNA) polymerase activity (9) has led to the suggestion that HBeAg and DNA polymerase activity may be identical (4). We performed the following experiments to examine the relationship between HBeAg, DNA polymerase, and Dane particles.

Portions of sera from a patient known to have circulating HBsAg, HBeAg, Dane particles, and DNA polymerase activity were layered onto 30% sucrose and centrifuged at 230,000 × g for 4 h at 4°C in a Beckman SW50.1 rotor (M. J. Tong, D. Stevenson, and I. Gordon, J. Infect. Dis., in press). The upper two-thirds of the sucrose fractions were removed and dialyzed against 0.01 M phosphate-buffered saline (pH 7.4). A portion of the dialyzed solution was concentrated with polyacrylamide gel, and the remainder was passed through an anti-HBsAg affinity column (13). After washing with phosphate-buffered saline, the column was eluted with sodium iodide. The washings and eluates were also concentrated with polyacrylamide gel. The pellets obtained after ultracentrifugation were either suspended in 100 μl of phosphate-buffered saline or treated with 0.5% Tween 80 (1). All specimens were examined by radioimmunoassay (Ausria II, Abbott Laboratories) to determine the presence or absence of HBsAg, for the incorporation of tritiated deoxyctydine and deoxyguanosine triphosphates into an acid-insoluble product to determine the presence or absence of any DNA polymerase activity (11), by rheophoresis to determine the presence or absence of HBeAg, and by electron microscopy to determine the presence or absence of Dane particles (T. D. Boyer, M. J. Tong, J. Rakela, and R. B. Reynolds, Am J. Dig. Dis., in press).

The results (Table 1) show that the sucrose fractions contained HBsAg and HBeAg but were devoid of significant DNA polymerase activity; the HBeAg and HBsAg were subsequently separated by affinity chromatography. Although HBeAg was not found in Dane-rich pellets that had been resuspended in phosphate-buffered saline, treatment of the pellets with Tween 80 resulted in the appearance of detectable HBeAg as well as an increase in DNA polymerase activity. Furthermore, HBeAg was not detected in similarly treated sera obtained from three HBsAg-positive patients, which originally contained Dane particles but were HBeAg negative and devoid of DNA polymerase activity.

Our results indicate that HBeAg exists in the serum in two forms. One form is freely circulating and can be physically separated from the HBsAg as well as from the HBV-specific DNA polymerase. The other form can be released from Dane particle-rich pellets by detergent treatment. Almeida et al. (1) showed that HBsAg may be separated from the core of the Dane particle by Tween 80 treatment, and release of HBeAg by this procedure suggests that this substance is present between the surface and the core of the hepatitis virion. Since anti-e serum has been demonstrated to agglutinate Dane particles (8), HBeAg may also be partly exposed on the surface of the Dane particle. The observations are explainable if HBeAg, in the bound form, is part of the matrix substance between the 7- and 8-nm HBsAg subunits on the surface of the viral particle (5) as well as between the coat and the inner HBcAg. Trepo et al., using immunofluorescent studies, have observed that HBeAg is present in the
cytoplasm of HBV-infected hepatocytes where HBsAg is also detected (3, 14). This raises the possibility that both HBeAg and HBsAg are synthesized in the cytoplasm where they are incorporated onto the core to form the complete virion, and free HBeAg in the circulation results from surplus synthesis as postulated for the 22-nm particles (3). It should be noted that this study did not exclude the possibility that the free HBeAg may be the polymerase protein whose enzyme activity has been destroyed. The separation of HBeAg from DNA polymerase activity after detergent treatment of the Dane-rich pellets would help clarify this question. Thus, our failure to release HBeAg from pellets obtained from HBeAg-negative sera may indicate defective synthesis of HBeAg by the hepatocyte, resulting in the production of incomplete viral particles (2). If so, the presence of circulating HBeAg may represent the formation of complete virions and may be indicative of potential HBV infectivity.

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LITERATURE CITED

TABLE 1. Results of sucrose density gradient centrifugation, affinity chromatography, and Tween 80 treatment of HBeAg-positive sera

<table>
<thead>
<tr>
<th>Specimen</th>
<th>HBsAg</th>
<th>HBeAg</th>
<th>DNA polymerase activity (cpm)</th>
<th>% Dane particles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original serum</td>
<td>+</td>
<td>+</td>
<td>396</td>
<td>3.8 (1,600)</td>
</tr>
<tr>
<td>Ultracentrifugation</td>
<td>+</td>
<td>+</td>
<td>68</td>
<td>2.4 (1,000)</td>
</tr>
<tr>
<td>Sucrose fraction, affinity chromatography</td>
<td>-</td>
<td>+</td>
<td>10</td>
<td>ND^b</td>
</tr>
<tr>
<td>Washing</td>
<td>-</td>
<td>+</td>
<td>123</td>
<td>ND</td>
</tr>
<tr>
<td>Eluate</td>
<td>+</td>
<td>-</td>
<td>1,441</td>
<td>28.2 (2,300)</td>
</tr>
<tr>
<td>Pellet</td>
<td>+</td>
<td>-</td>
<td>12,443</td>
<td>ND</td>
</tr>
<tr>
<td>Phosphate-buffered saline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tween 80</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

^a The numbers in parentheses represent the total sum of tubular forms, 22-nm particles, and Dane particles that were counted with electron microscopy.

^b ND, Not done.