Hepatitis B core antigen synthesised in *Escherichia coli*: its use for antibody screening in patients attending a clinic for sexually transmitted diseases

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**Summary**

Hepatitis B core antigen (HBcAg) synthesised in *Escherichia coli* by recombinant DNA techniques was compared with HBcAg prepared from infected liver tissue. The two antigens were used in radioimmunoassays (RIA) to detect antibody to HBcAg (anti-HBc) in sera from patients attending a clinic for sexually transmitted diseases. Out of 2151 sera tested, 260 were anti-HBc positive with both HBcAg preparations but seven were positive with the liver-derived antigen alone. Reasons for these discrepant results are discussed. The slight loss of sensitivity of the anti-HBc RIA using *E. coli* HBcAg was not considered significant when compared with the potential advantages of a synthetic antigen.

The presence of other hepatitis B markers in the 267 anti-HBc positive sera was determined: 25 contained HBsAg, 220 anti-HBs and, of the 22 that were HBsAg/anti-HBs negative, 12 contained anti-HBe. In the 10 remaining sera, anti-HBc was the only hepatitis B marker that could be found.

**Introduction**

Synthesis of the antigenic components of hepatitis B virus (HBV) in *Escherichia coli* has been achieved through the application of recombinant DNA techniques. Only limited amounts of hepatitis B surface antigen (HBsAg) have been produced (MacKay *et al.*, 1981), but hepatitis B core antigen (HBeAg) is more readily made (Burrell *et al.*, 1979) and hepatitis B e antigen (HBeAg) can be derived from it (MacKay, Lees & Murray, 1981).

The availability of *E. coli*-derived HBcAg in working amounts permits its use in radioimmunoassay (RIA) for antibody to HBeAg (anti-HBe). Initial studies indicated that the bacterial antigen was suitable for this purpose (Peutherer *et al.*, 1981; Stahl *et al.,* 1982). We now report a study in which HBeAg synthesised in *E. coli* was used to determine the prevalence of anti-HBe in patients attending a clinic.

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for sexually transmitted disease (STD). This group is known to have a relatively high frequency of HBV infection (Szmuness et al. 1976). The results of anti-HBc screening with E. coli-derived HBeAg were compared to those obtained with HBeAg prepared from human liver, and the presence of other HBV markers was determined in reactive samples.

MATERIALS AND METHODS

Survey sera

Two thousand one hundred and fifty one sera from unselected male and female patients attending a STD clinic were collected between August 1978 and January 1979. Some of the patients were tested more than once during this period.

Hepatitis B core antigen

An extract of E. coli containing HBeAg was kindly provided by Professor K. Murray, Dept. of Molecular Biology, University of Edinburgh. An extract of human liver containing HBeAg was prepared as described previously (Cohen & Cossart, 1977).
Anti-HBc screening with HBcAg from E. coli

All sera

Anti-HBc

Positive

n = 267

Negative

n = 1884

HBsAg

Positive

n = 25

Negative

n = 242

Anti-HBs

Negative

n = 22

Positive

n = 220

HBeAg

Anti-HBe

Anti-HBc IgM

Fig. 2. Flow chart of study and results.

Serological tests for hepatitis B virus

All sera were screened for anti-HBc using both the E. coli and liver-derived HBcAg preparations. A ‘competitive’ solid phase RIA method was followed (Cohen, Hewish & Mortimer, 1981) but with a few modifications. Instead of polystyrene tubes, 6.5 mm polystyrene beads (Northumbria Biologicals Ltd.) were used as the solid phase. E. coli-derived HBcAg was bound indirectly via a coating of anti-HBc IgG because initial experiments had shown that the bacterial antigen was bound more efficiently this way (Fig. 1). The assay procedure was the same with both antigens. A 20 μl volume of test sample was mixed with 180 μl of ¹²⁵I-labelled-anti-HBc IgG, diluted to give approximately 80000 c.p.m., and incubated for 24–48 h at room temperature. Sera inhibiting 80% or more of label binding were considered positive. Those inhibiting 60–79% of label binding were considered weakly positive and those inhibiting < 60% of label binding were considered negative.

Anti-HBc positive sera were tested for other markers of HBV infection as indicated in the flow chart (Fig. 2). HBsAg, anti-HBs, HBeAg and anti-HBe were determined by commercially available test kits (Abbott Laboratories). Anti-HBc IgM was measured by ‘M-antibody capture’ RIA (Mortimer et al. 1981).
Table 1. Correlation of anti-HBc results using two HBcAg preparations

<table>
<thead>
<tr>
<th></th>
<th>Anti-HBc positive</th>
<th>Anti-HBc negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver-HBcAg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>260</td>
<td>7</td>
<td>267</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>1884</td>
<td>1884</td>
</tr>
<tr>
<td>Total</td>
<td>260</td>
<td>1891</td>
<td>2151</td>
</tr>
</tbody>
</table>

Table 2. Results on seven sera giving discrepant results in anti-HBc assays

<table>
<thead>
<tr>
<th>Liver-HBcAg</th>
<th>E. coli - HBcAg</th>
<th>HBsAg</th>
<th>Anti-HBs</th>
<th>Anti-HBe</th>
</tr>
</thead>
<tbody>
<tr>
<td>67.1</td>
<td>18.6</td>
<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>72.0</td>
<td>40.6</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>62.0</td>
<td>9.3</td>
<td></td>
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<td>+</td>
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<td>70.7</td>
<td>43.9</td>
<td></td>
<td>+</td>
<td>-</td>
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<td>70.6</td>
<td>53.1</td>
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<td>+</td>
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<td>60.7</td>
<td>20.1</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>62.5</td>
<td>41.1</td>
<td></td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

RESULTS

Serological tests for hepatitis B virus

The main results from the testing scheme used in this study are given in the flow chart (Fig. 2).

Anti-HBc

The correlation between results for anti-HBe obtained with the two HBcAg preparations is shown in Table 1. Results on seven of 2151 sera were discrepant: all seven were weakly anti-HBe positive with liver-derived HBcAg, but negative with *E. coli* HBcAg (Table 2). All contained at least one other marker of HBV infection.

The discrimination between positive and negative anti-HBe reactions by the two HBc antigens is illustrated in figure 3. The liver HBcAg gave more strongly positive, but fewer weakly positive reactions than the *E. coli* HBcAg. The bacterial antigen, however, gave more clearly negative reactions (< 10% inhibition of 125I anti-HBe binding) and fewer 'sub cut-off' reactions (40–60% inhibition) than the liver antigen.

HBcAg and Anti-HBs

The 267 positive sera could be divided into three categories according to their HBsAg/Anti-HBs status. Twenty-five were HBsAg positive, 220 anti-HBs positive, and 22 were negative for HBsAg and anti-HBs.
Anti-HBc screening with HBcAg from E. coli

Fig. 3. Discrimination between positive and negative reactions in anti-HBc RIA using two HBcAg preparations. (a) Liver HBcAg; (b) E. coli HBcAg.

HBeAg and anti-HBe

There was a sufficient volume available for HBeAg and anti-HBe tests on 21 of the 25 HBsAg positive sera (Table 3). Four were HBeAg positive, 16 anti-HBe positive and one was HBeAg/anti-HBe negative. The 22 HBsAg/anti-HBs negative sera were tested for anti-HBe. Ten were positive and 12 were negative.

Anti-HBe IgM

Anti-HBe IgM assays were carried out on the 25 anti-HBe positive sera which were HBsAg positive and the 22 anti-HBe positive sera which were HBsAg/anti-HBs negative (i.e. anti-HBe/anti-HBs positive specimens were not tested). Large amounts of anti-HBe IgM were detected in three HBsAg positive sera but in none of the HBsAg negative sera. These three sera were also HBeAg positive (Table 3). Small amounts of anti-HBe IgM were found in three other sera, of which two were HBsAg positive, and one HBsAg/anti-HBs negative. These three sera were anti-HBe positive (Table 3).
Table 3. HBsAg, anti-HBe and anti-HBc IgM results on anti-HBc positive sera with and without HBsAg

<table>
<thead>
<tr>
<th></th>
<th>Number of sera</th>
<th>Anti-HBc IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HBeAg</td>
<td>Anti-HBe</td>
</tr>
<tr>
<td>HBsAg positive</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>n = 25</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>HBsAg/anti-HBs</td>
<td>NT</td>
<td>+</td>
</tr>
<tr>
<td>negative n = 22</td>
<td>NT</td>
<td>-</td>
</tr>
</tbody>
</table>

* Number in brackets is number with > 10 units anti-HBe IgM (i.e. strongly positive). NT denotes not tested.

**DISCUSSION**

Comparison of *E. coli* and liver-derived HBcAg

A close correlation was obtained between results with the two antigens used to screen for anti-HBc. The only discrepant results were on seven sera that were weakly positive for anti-HBc positive with liver HBcAg but negative with *E. coli* HBcAg. We consider that these were true positive anti-HBc reactions because all seven sera contained other hepatitis B antibodies.

The seven false negative results with the *E. coli* HBcAg suggest that anti-HBc RIA with this antigen is slightly less sensitive than anti-HBc RIA with HBcAg of human origin. This suggestion is supported by a comparison of percentage inhibition values obtained with the two antigens in the competitive RIA used for anti-HBc used in this study. The inhibition of 125I anti-HBc binding was generally lower when *E. coli* HBcAg was on the solid phase than when liver HBcAg was used. The *E. coli* antigen, however, discriminated better between anti-HBc positive and negative sera. It detected 260 out of 267 positive samples, and we believe that the slight loss of sensitivity in tests with it is negligible when compared with the potential advantages of a synthetic antigen as a diagnostic reagent.

The differences in antibody binding characteristics of the two antigens are probably due to incomplete identity between *E. coli* and liver HBcAg. Although complete identity between the two antigens has been shown by immunodiffusion (Stahl et al. 1982) minor antigenic differences, undetectable by this test, may exist. They could be related to the altered amino acid sequence at the N-terminus of the bacterial HBcAg polypeptide (Stahl et al. 1982). A study of these antigenic differences using monoclonal antibodies would be of interest.

Alternatively, the discrepant anti-HBe results may have been due to the way in which the two antigens were bound to the solid phase. The liver HBcAg was bound directly whereas the *E. coli* HBcAg was bound indirectly by anti-HBe IgG. This may have resulted in a difference in the presentation of the antigens for competitive binding of anti-HBe which is the basis of the assay used in this study.

In spite of the minor discrepancies this study confirms that bacterial synthesis of HBcAg results in a satisfactory reagent for hepatitis B serology. Antigen from *E. coli* discriminates well between specimens with and without anti-HBc and offers...
Anti-HBc screening with HBcAg from *E. coli* several advantages over HBcAg prepared from human liver. The dearth of HBcAg from human or other primate sources has restricted the use of anti-HBe assays, but the potentially unlimited supply of HBcAg from *E. coli* would allow anti-HBe testing to be applied to, for example, blood donor screening. The genetically homogeneous origin of an *E. coli* antigen means that a standard HBcAg could be prepared. This would permit HBcAg from all sources to be compared and facilitate the standardization of anti-HBe assays. An additional advantage of *E. coli* HBcAg is that potentially hazardous procedures involved in extracting the antigen from post-mortem liver or from serum rich in Dane particles can be avoided. The synthesis of HBV antigens in *E. coli* is based on materials lacking the genetic capacity to produce complete, infectious virus (Murray *et al.* 1981).

**Anti-HBc screening of STD clinic patients.**

The prevalence of anti-HBe in this survey of STD clinic patients was 12·5% (267/2151). This compares with an anti-HBe prevalence in blood donors of 1·3% in North London (Tedder *et al.* 1980) and 0·7% in Bristol (Archer, Cohen & Mortimer, 1983). The higher prevalence of anti-HBe in STD clinic patients reflects the increase frequency of HBV infection in sexually promiscuous groups (Szmuness *et al.* 1976). The infection is particularly common amongst male homosexuals (Coleman, Waugh & Dalton, 1977). Thus in a group of STD patients that included 47% male homosexuals, Tedder and colleagues found anti-HBe in 44% (58/133). This was higher than the 12·5% prevalence of anti-HBe in the present survey population, most of whom were heterosexual. Other epidemiological factors bearing on the distribution of anti-HBe, such as country of origin and history of jaundice, have not been analysed.

Of 2151 sera tested, 25 (2·1%) were found to be HBsAg positive. This compares with an HBsAg prevalence of 0·2% in North London blood donors (Barbara *et al.* 1977). It is possible that a few sera containing HBsAg remained undetected in this survey, as only sera positive in the initial anti-HBe screening tests were examined for HBsAg. Patients in the early incubation phase of HBV infection would have been missed because, at this stage, HBsAg is present in the absence of anti-HBe.

By contrast, blood collected from patients convalescent from HBV infection may be anti-HBe positive but HBsAg negative. Such material is capable of transmitting hepatitis B (Dike, 1981).

Four of the 25 HBsAg positive sera were also positive for HBeAg. Three of these were strongly positive for anti-HBe IgM, indicating recent HBV infection. Chronic infection was suggested in the fourth, HBsAg and HBeAg positive serum, which was anti-HBe IgM negative.

Of 267 patients with anti-HBe, 220 had anti-HBs. As only anti-HBe positive sera were tested it is not known how many sera without anti-HBe were anti-HBs positive. The study of Tedder and co-workers found no STD clinic patients with anti-HBs in the absence of anti-HBe.

There were 22 anti-HBe positive sera that were HBsAg and anti-HBs negative. This pattern of reaction suggests recovery from acute HBV infection, when HBsAg has been cleared but anti-HBs has not yet been produced. Anti-HBe is frequently detected at this stage, and, in the present study 10 out of 22 sera in this category were anti-HBe positive. One of the ten sera was weakly positive for anti-HBe IgM.
also suggesting recent infection. The remaining HBsAg and anti-HBs negative sera were negative for both anti-HBe and anti-HBc IgM. Anti-HBc (presumably anti-HBc IgG) was thus present as the only marker of HBV infection. These reactions are likely to be specific since they were obtained with both *E. coli* and liver HBcAg, and it is concluded that anti-HBc IgG may be the sole marker of a past HBV infection in some patients who have lost, or never made, detectable anti-HBs and anti-HBe.

We would like to thank Professor K. Murray and Biogen N. V. for supplying *E. coli* HBcAg. We are grateful to Dr E. M. Vandervelde for advice and Mr R. A. Hewish for technical assistance.

**REFERENCES**


