Radioimmunoassay and Some Properties of Human Antibodies to Hepatitis B Core Antigen

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SUMMARY

A solid-phase radioimmunoassay for antibodies to hepatitis B core antigen (anti-HBc) is described. Polystyrene beads coated with anti-HBc, hepatitis B core antigen prepared from pooled sera of humans infected with hepatitis B virus (HBV) and 125I-labelled anti-HB, were used for the test.

Distinct patterns of development and changes of anti-HBc and their immunologic properties are all related to variations of other markers specific for HBV infections. Knowledge concerning the detailed features of the immune response to hepatitis B core antigen may provide deeper insight into the pathogenesis of HBV infections.

INTRODUCTION

The putative hepatitis B virus, commonly designated as the Dane particle (Dane, Cameron & Briggs, 1970) consists of a nucleocapsid 27 nm in diameter within a lipoprotein shell. Distinct antigenic determinants are associated with the outer surfaces of the lipoprotein envelope and the nucleocapsid (Almeida, Rubenstein & Stott, 1971). These determinants are referred to as hepatitis B surface antigen (HBsAg) and hepatitis B core antigen (HBcAg) respectively. Several techniques have been developed for the assay of antibody to HBsAg (anti-HBs): complement fixation (Hoofnagle, Gerety & Barker, 1973), immune adherence haemagglutination (Tsuda et al. 1975), immunofluorescence (Brzosko et al. 1975), counter-immunoelectrophoresis tests (cf. Howard & Zuckerman, 1977), and radioimmunoassays using either HBsAg containing tritiated DNA (Robinson & Greenman, 1974; Moritsugu et al. 1975; Greenman, Robinson & Vyas, 1975; Howard & Zuckerman, 1977) or 125I-labelled anti-HBc (Purcell et al. 1973; Vyas & Roberts, 1977).

Anti-HBc develops regularly during type B hepatitis and is an indicator of either recent acute infection or of chronic infection by hepatitis B virus (Hoofnagle et al. 1973, 1974; Krugman et al. 1974; Hoofnagle, Gerety & Barker, 1975; Szmuness et al. 1976). The titre of anti-HBc in sera of HBsAg carriers appeared to be unrelated to the concentration of Dane particles determined directly by electron microscopy or estimated indirectly by testing the sera for HBsAg-specific DNA polymerase activity or for e antigen (HBcAg) (Takahashi et al. 1976; Imai et al. 1976), an additional soluble antigen specifically associated with hepatitis B virus (HBV) infections (Magnius & Espmark, 1972) and an apparent marker for the infectivity of HBcAg-positive sera.

We describe here a sensitive solid-phase radioimmunoassay for anti-HBc and its appli-
cation to the study of anti-HBc in individuals infected by HBV. Our results suggest that anti-HBc present in HBcAg-positive sera differs qualitatively from anti-HBc in sera containing antibodies to HBcAg (anti-HBc), and that the magnitude of quantitative changes in the titre of anti-HBc with time may be of value in predicting the probable outcome of infections by HBV.

METHODS

Preparation of the HBcAg reagent. Sera positive for HBcAg were centrifuged at 37000 g for 16 h to pellet the Dane particles. In some cases, the sera were first precipitated with 6% (w/v) polyethylene glycol, and the pellets containing Dane particles (Neurath & Strick, 1977) were resuspended in 0.01 M-tris (hydroxymethyl) aminomethane-0.14 M-NaCl-0.02% NaN3 (TS) in 1/5 of the original serum volume and then centrifuged as above. The pellets containing Dane particles were suspended in TS in 1/100 of the original serum volume and pronase (100 μg/ml) was added. The mixtures were incubated for 1 h at 37 °C. Solid KBr was added to a buoyant density of 1.30 g/ml and the mixtures were transferred into centrifuge tubes (3 to 8 ml/tube) for either the SW 25.2 or SW 25.1 rotors (Spinco Division, Beckman Instruments, Palo Alto, California). A gradient of KBr (density 1.28 to 1.15 g/ml) was layered on top of the mixtures. The tubes were centrifuged at 25000 rev/min for 24 h. Fractions with a density of 1.20 to 1.24 g/ml which contained most of the HBcAg and the Dane particles were pooled, dialysed against TS and then centrifuged at 37000 g for 16 h. The pellets were resuspended in 1 ml of TS and treated with 50 μg of pronase for 1 h at 37 °C and then layered on top of a 7.5 to 20% (w/w) sucrose gradient in a centrifuge tube for the SW 65 rotor. The pellet obtained after centrifuging at 35000 rev/min for 2 h was resuspended in 5 ml of 0.1 M-NH4Cl-0.08 M-MgCl2-0.25% mercaptoethanol-0.5% Nonidet P40 (pH 7.6), incubated for 1 h at 37 °C and stored at 4 °C. No loss of HBcAg activity was observed after storage for 4 months. The properties of HBcAg will be described in a separate paper (Neurath, Huang & Strick, 1978).

Radioimmunoassay for anti-HBc. Polystyrene beads (diam. 6 mm) were immersed in a solution of anti-HBc [100 μg IgG/ml in 0.1 M-tris (hydroxymethyl) aminomethane, pH 8.8] prepared from an anti-HBc-positive serum as described before (Neurath & Strick, 1977). After several hours at room temperature, the solution of anti-HBc was removed, the beads were washed several times with TS and then immersed in a solution of bovine haemoglobin (10 mg/ml in TS). After standing overnight at 4 °C, the beads were washed with TS, dried and stored at 4 °C.

Anti-HBc from anti-HBc, and HBcAg-positive sera were labelled with Na125I or with Na131I as described before (Neurath & Strick, 1977).

For routine determinations of anti-HBc, proper dilutions (0.4 ml) of test samples in sheep serum diluted 1:5 with TS were mixed with 1 μl of the HBcAg reagent and incubated for 30 min at 37 °C. Negative (1:5 sheep serum) and positive (1:5 sheep serum + 1 μl of the HBcAg reagent) controls were included with each set of test samples. Anti-HBc-coated beads were added and the incubation was continued for 16 h at room temperature. The beads were washed with TS and then incubated for 2 h at 37 °C with 0.3 ml of 1:5 sheep serum and with 0.1 ml of the labelled anti-HBc, (200000 ct/min). Finally, the beads were washed with TS and counted in a γ-counter. Radioactive counts corresponding to the negative control were subtracted from all results.

To study the competition for determinants on HBcAg between unlabelled anti-HBc, 125I- and 131I-labelled anti-HBc, beads were first incubated overnight at room temperature with the HBcAg reagent (1 μl in 0.4 ml of 1:5 sheep serum). The beads were washed with
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TS, and a mixture of unlabelled and labelled (200000 ct/min for each label) anti-HBc in 1:5 sheep serum was added. The beads were incubated for 2 h at 37 °C, washed with TS and counted in a two-channel γ-counter. All results were corrected for spillover of 125I-counts into the channel for counting 131I.

Other methods. Isoelectric focusing was performed as described before (Neurath & Strick, 1977). HB,Ag and antibodies to HB,Ag (anti-HBc) were determined by radioimmunoassays using commercial reagents (Abbott Laboratories, North Chicago, Illinois). Rheophoresis was used to detect HB,Ag and anti-HBc (Neurath & Strick, 1977). Serum glutamic–pyruvic transaminase (SGPT) was tested spectrophotometrically using the Enztrate test kit (Beckman Instruments, Fullerton, California) and following the instructions of the manufacturer. Sera were collected from patients and personnel of haemodialysis units in biweekly to 1 month intervals.

To study the class specificity of anti-HBc, 40 μl samples of sera diluted with TS to 1 ml were applied to 2.5 ml columns of insolubilized antibodies to human immunoglobulins. The columns were prepared by binding the IgG fractions from antisera to human immunoglobulins (Behring Diagnostics, Somerville, New Jersey) to CNBr–Sepharose (Pharmacia, Uppsala, Sweden). The columns were incubated for 30 min at 37 °C and 1 h at 4 °C and then washed at 4 °C with 6 ml of TS supplemented with NaCl (0.5 M). The adsorbed immunoglobulins were eluted at 24 °C with 6 ml of 0.2 M-NaHCO3–0.5 M-NaCl (pH 10.9).

RESULTS

Specificity and relative sensitivity of the radioimmunoassay for anti-HBc

The basis of the radioimmunoassay is the inhibition by anti-HBc in serum test samples of the attachment of HB,Ag to plastic beads coated with anti-HBc. The quantity of HB,Ag bound to the beads is determined by measuring the amount of 125I-labelled anti-HBc adsorbed to the beads during a subsequent incubation step after they had been washed to remove all unattached HB,Ag and unlabelled anti-HBc from the samples. To determine the optimum quantity of HB,Ag for the test, serial twofold dilutions of the HB,Ag reagent in sheep serum diluted fivefold with TS were incubated with the beads overnight at 24 °C and the relative quantity of bound HB,Ag was determined. The quantity of 125I-labelled anti-HBc bound to the beads increased non-linearly with decreasing dilution of HB,Ag (Fig. 1). One μl of the HB,Ag reagent per test (corresponding to a dilution of 1:400) was used in subsequent experiments. Incubation of the beads with HB,Ag instead of HB,Ag did not cause subsequent attachment of the radioactive antibody. Addition of anti-HBc to HB,Ag inhibited the binding of 125I-labelled anti-HBc to the beads (Fig. 2). Some sera from carriers of HB,Ag tested in our experiments caused a 50% inhibition at dilutions up to 1:51000. The slopes of dose response curves varied for different sera, an observation which will be discussed later.

To evaluate the relative sensitivity of the test for anti-HBc, a panel of sera supplied by the Bureau of Biologies, Food and Drug Administration and used for evaluation of the sensitivity of tests for HB,Ag was screened for anti-HBc. All sera were tested at a 1:16 dilution. Results in Table 1 show that the radioimmunoassay for anti-HBc is less efficient in screening out HB,Ag carriers from blood donors than current tests for HB,Ag. However, the method identifies additional individuals whose serum may contain HBV although negative for HB,Ag by current test methods (Table 2).

To further evaluate the specificity of the radioimmunoassay for anti-HBc sera from patients with HB,Ag-negative alcoholic liver cirrhosis (5), acute non-A non-B hepatitis
Fig. 1. Radioimmunoassay of HBsAg. Relationship between 125I-labelled anti-HBs bound to polystyrene beads and the dilution of HBsAg.

Fig. 2. Radioimmunoassay inhibition test of anti-HBc. Dose response curves for a serum containing HBsAg (○-----○) and anti-HBc (●-●), respectively. Horizontal broken line indicates the radioactivity corresponding to a positive control containing HBsAg but no anti-HBc.

Table 1. Detection of anti-HBc in a panel of HBsAg reference sera

<table>
<thead>
<tr>
<th>Relative concentration of HBsAg in serum as indicated by the supplier of the panel</th>
<th>Frequency of anti-HBc detection at a serum dilution of 1:16*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Reactive by all methods including agar gel diffusion</td>
<td>3/3</td>
</tr>
<tr>
<td>B. Reactive by counter-electrophoresis, reversed passive haemagglutination and radioimmunoassay</td>
<td>5/5</td>
</tr>
<tr>
<td>C. Reactive only by reversed passive haemagglutination and radioimmunoassay</td>
<td>6/9</td>
</tr>
<tr>
<td>(C). Only occasionally positive by third generation (C) test methods</td>
<td>1/2</td>
</tr>
<tr>
<td>D. Non-reactive by current test methods but containing low levels of HBsAg</td>
<td>1/2</td>
</tr>
<tr>
<td>N. Non-reactive by current test methods</td>
<td>2/4</td>
</tr>
</tbody>
</table>

* All sera inhibited the binding of 125I-labelled anti-HBc by more than 50%.

(Prince et al. 1974) (3), and acute hepatitis A (5) were tested at a dilution of 1:16. Only two of the sera had anti-HBc, and these two were from hepatitis A virus-infected children from a school of the mentally retarded, i.e., from an environment where hepatitis B is also prevalent. Normal human sera and animal sera tested undiluted inhibited the attachment of 125I-anti-HBc by about 40 to 60%. The nature of this effect remains unknown. However, some human sera (tested undiluted or 1:4 diluted) had a higher inhibitory effect, suggesting the presence of low levels of anti-HBc.
Table 2. Detection of anti-HBc in a group of HBsAg-negative sera

<table>
<thead>
<tr>
<th>Characterization of serum based on previous results (Szmuness et al. 1976)*</th>
<th>Frequency of anti-HBc detection by radioimmunoassay at a serum dilution of 1:16</th>
<th>Minimal inhibition of 131I-anti-HBc attachment observed in group†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive for both anti-HBc and anti-HBo</td>
<td>11/11</td>
<td>40%</td>
</tr>
<tr>
<td>Negative for anti-HBc</td>
<td>6/6</td>
<td>42%</td>
</tr>
<tr>
<td>Positive for anti-HBo</td>
<td>7/16</td>
<td>20%</td>
</tr>
</tbody>
</table>

* Anti-HBc was detected by complement fixation tests.
† The highest inhibition observed with 2 out of 32 normal human sera tested at 1:16 dilutions was 13.3%. An inhibition equal to or higher than 20% was considered specific for anti-HBc.

Table 3. Prevalence of anti-HBc in serum (negative for both HBsAg and anti-HBc) of patients and staff members of haemodialysis units

<table>
<thead>
<tr>
<th>Characterization of the test group</th>
<th>Frequency of anti-HBc detection at a serum dilution of 1:16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients haemodialysed for more than 3 years</td>
<td>12/25</td>
</tr>
<tr>
<td>Patients haemodialysed for less than 1 year</td>
<td>1/19</td>
</tr>
<tr>
<td>Staff members employed for less than 1 year</td>
<td>1/22</td>
</tr>
</tbody>
</table>

Development and changes in anti-HBc in the serum of selected individuals infected with HBV

Hepatitis B is prevalent among patients and staff members of haemodialysis units. The radioimmunoassay test for anti-HBc identified additional individuals who were infected with HBV but whose serum was negative for both HBsAg and anti-HBc, and did not show elevated levels of SGPT (Table 3). Considering the frequency of positive tests for each HBsAg, anti-HBc, and anti-HBc, at least 75% of patients treated for more than 3 years were infected with HBV. The high rate of HBV infections in haemodialysis units and the long-term surveillance of patients and staff members allowed us to select individuals recently infected with HBV who showed distinct patterns of anti-HBc development in relation to other markers for HBV infection. The results summarized in Fig. 3 indicate that: (1) unless present at the initiation of surveillance (panels 5, 6, 7), anti-HBc appeared abruptly at titres ≥ 1:27; (2) the initiation of synthesis of anti-HBc coincided approximately with the first signs of liver damage as indicated by elevated levels of serum SGPT (panels 1, 2, 3, 4, 8, 9, 10); (3) clearance of HBsAg from serum and/or detection of anti-HBc, usually occurred during a decline of anti-HBc levels (panels 1 to 7); (4) a low rate of clearance of anti-HBc (panel 5) and a prolonged period in which levels of anti-HBc increased (panels 8 to 10) seemed to be associated with sporadic and prolonged liver damage, respectively; (5) HBsAg appeared in serum during a time interval in which titres of anti-HBc increased (panels 8 to 10); (6) infection with HBV is not necessarily associated with even transient presence of HBsAg (detectable by radioimmunoassay) in serum (panel 4); (7) the fast clearance rate of anti-HBc, from serum (panels 2, 3, 4, 6, 7) corresponded to an anti-HBc half-life of 16 to 53 days; (8) after clearance of HBsAg from serum and before anti-HBc becomes detectable, anti-HBc represents the only marker for HBV infection (panels 1, 2, 3, 5, 6, 7, 8).

In order to determine the class of anti-HBc appearing early in the course of infection, samples of the first specimen positive for anti-HBc (panel 9, Fig. 3) were chromatographed
Fig. 3. Development of anti-HBc in relation to other markers of infection by HBV in patients (panels 5 to 10) and staff members (panels 1 to 4) of haemodialysis units. All sera were tested for anti-HBc at a 1:52 dilution. Sera negative for anti-HBc were retested at a 1:8 dilution and were again found negative in all cases. The 50% inhibition end-points were determined from dose response curves (see Fig. 2) and plotted on Fig. 3 (○-○). Short perpendicular lines at the ends of the bars representing different HBV markers indicate positivity at the start or termination of surveillance. Position of bars along vertical axis has no meaning. □, Anti-HBc; □, HBsAg; ■, anti-HBc; ■, elevated SGPT; □, HBsAg.
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Table 4. Association of early anti-HBc with distinct immunoglobulin classes

<table>
<thead>
<tr>
<th>Type of immunosorbent column*</th>
<th>Anti-HBc titre† of unadsorbed immunoglobulins</th>
<th>Adsorbed immunoglobulins subsequently eluted at pH 10.9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-IgG</td>
<td>1:24</td>
<td>1:10</td>
</tr>
<tr>
<td>Anti-IgA</td>
<td>1:23</td>
<td>1:9</td>
</tr>
<tr>
<td>Anti-IgM</td>
<td>1:3</td>
<td>1:21</td>
</tr>
</tbody>
</table>

* Immunodiffusion tests with the unadsorbed and the adsorbed and subsequently eluted proteins revealed that each immunosorbent column specifically and completely adsorbed the immunoglobulins of the proper class. Anti-IgD and anti-IgE columns adsorbed very little anti-HBc, probably non-specifically.
† Corresponding to 50% inhibition endpoints determined from anti-HBc dose response curves (Fig. 2).

Table 5. Comparison of slopes of anti-HBc dose response curves from HBcAg- and anti-HBc-positive sera*

<table>
<thead>
<tr>
<th>Characterization of anti-HBc</th>
<th>Magnitude of maximum slope (ct/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBcAg-positive serum</td>
<td>150; 220; 230; 250; (mean†: 213 ± 22)</td>
</tr>
<tr>
<td>Anti-HBc-positive serum</td>
<td>700; 450; 370; 300; (mean‡: 455 ± 87)</td>
</tr>
<tr>
<td>F((ab)_2) fragment from:</td>
<td></td>
</tr>
<tr>
<td>HBcAg-positive serum</td>
<td>150 (173)†</td>
</tr>
<tr>
<td>Anti-HBc-positive serum</td>
<td>350 (410)‡</td>
</tr>
</tbody>
</table>

* The slopes reflect the avidity of antibodies (Celada, Schmidt & Strom, 1969).
† The difference between the two means is statistically significant (t test, P < 0.01).
‡ Values obtained with two different batches of anti-HBc-coated beads.

on columns of insolubilized antibodies to the distinct classes of human immunoglobulins. The unadsorbed, and the adsorbed and subsequently eluted immunoglobulins were tested for anti-HBc. Results summarized in Table 4 show that anti-HBc activity was associated predominantly with IgM. In a specimen taken from the same patient 106 days later, anti-HBc was detected mostly in IgG as determined by the same technique.

Qualitative differences between anti-HBc apparently related to the presence of either HBcAg or anti-HBc in individual sera

Differences in anti-HBc dose response curves were observed between sera positive for HBcAg and anti-HBc, respectively (Fig. 2). Similar results were obtained with three additional randomly selected pairs of HBcAg- and anti-HBc-positive sera and with F(\(ab\)_2) fragments prepared from IgG isolated from two of the respective sera (Table 5). In order to explain the observed differences in the slope of the dose response curves, anti-HBc IgG prepared from HBcAg- and anti-HBc-positive sera were labelled with \(^{125}\text{I}\) and \(^{131}\text{I}\) respectively. Experiments using beads coated with HBcAg revealed that the \(^{125}\text{I}\)- and \(^{131}\text{I}\)-labelled anti-HBc competed for the same antigenic sites on HBcAg and that the \(^{125}\text{I}\)-labelled antibodies were bound preferentially when an excess of both radioactive antibodies was added to the beads. This suggested that anti-HBc, from anti-HBc-positive sera may have a higher average affinity for antigenic sites on HBcAg than corresponding antibodies from HBcAg-positive sera. Therefore, it was expected that addition of unlabelled anti-HBc to HBcAg-coated beads suspended in a mixture of \(^{125}\text{I}\)- and \(^{131}\text{I}\)-labelled anti-HBc (added in constant
Fig. 4. Results of three-way competition tests between anti-HB, present in individual sera (tested at dilutions of 1:27 and 1:270) positive for either HB,Ag (○) or anti-HB, (●), 131I-labelled anti-HB, (isolated from an anti-HB, positive serum) and 125I-labelled anti-HB, (isolated from an HB,Ag positive serum). Both labelled anti-HB, were added at fixed ratios in identical amounts for each test. Circles with bars on the right side of individual plotted experimental results correspond to the mean values. The length of the bars indicates the magnitude of the standard error of the mean.

quantities) would suppress the binding of each of the labelled antibodies unequally, depending on the origin of the cold anti-HB,. This would result in a change of the ratio of 125I/131I anti-HB, bound to the beads as compared with the proportion of each labelled anti-HB, adsorbed to the beads when cold anti-HB, was absent. Indeed, additions of 2- to 128-fold dilutions of an HB,Ag- and an anti-HB, positive serum (each positive for anti-HB,) to identical mixtures of labelled anti-HB, altered the 125I/131I ratio of bound radioactivity 1.04- to 2.2-fold and 0.35- to 0.93-fold, respectively. The lower numbers in each case corresponded to lower serum dilution and vice versa. Based on these results, additional anti-HB,-positive (21) and HB,Ag-positive (69) sera were tested in the same way at two distinct dilutions. The results (Fig. 4) indicate a statistically significant difference (P < 0.01 for a t test of significance between two sample means) between anti-HB, in HB,Ag- and anti-HB, positive sera.

The preferential binding of 125I-labelled antibodies as compared to 131I-labelled immunoglobulins was not due to the distinct isotopes used. When each antibody was labelled with 125I, the tagged immunoglobulin from the anti-HB,-positive serum was less efficiently displaced by unlabelled anti-HB, from HB,Ag-coated beads than was the 125I-anti-HB, from HB,Ag-positive serum.

To determine whether the observed differences in the behaviour of anti-HB, depending on their origin were reflected in their physical properties, HB,Ag- and anti-HB,-positive sera from long-term HB,Ag carriers were submitted to isoelectric focusing. Results in Fig. 5 indicate that regardless of its origin, anti-HB, was heterogeneous with respect to charge
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and therefore probably polyclonal. The distribution of anti-HBc in the pH gradient was within the pH range of 7 to 9.75. The largest portion of anti-HBc was recovered in fractions with a pH between 8.25 and 9.25.

DISCUSSION

Brzosko et al. (1975) detected anti-HBc predominantly in IgG by an indirect immunofluorescent test when they examined sera collected during the first week of overt clinical illness from patients acutely infected with HBV. These authors did not notice a shift in the class specificity of anti-HBc from IgM to IgG during the course of illness and concluded that HBV infections may differ from other viral and non-viral infection with respect to development of the immune response to the particular antigens. Our limited results obtained by methods more amenable to quantitative evaluation than immunofluorescence, indicate a switch in anti-HBc production from IgM to IgG, as would be expected (Svehag, 1966).

As noticed before (Hoofnagle et al. 1974; Krugman et al. 1974; Hoofnagle et al. 1975), anti-HBc becomes detectable in serum at or close to the time during which clinical symptoms and/or abnormal levels of liver enzymes in serum are evident, i.e., much before appearance of a humoral or cellular immune response to HBcAg (Vyas et al. 1975). The SGPT activity returned to normal levels in serum usually during a decline of anti-HBc titres (Fig. 3). In other individuals, a prolonged period during which titres of anti-HBc increased was associated with prolonged liver damage. These results suggest a correlation, not necessarily causal, between liver injury, the synthesis of HBcAg and the immune response to it.

In some individuals with probably good prognosis for recovery, anti-HBc was cleared rapidly from serum in agreement with previous results (Krugman et al. 1974; Hoofnagle et al. 1975). The relatively short half-life of anti-HBc (16 to 53 days) as compared with the average half-life of IgG of 21 days (Stevenson, 1974) suggests elimination of the immunologic stimulus, i.e., cessation of the synthesis of HBcAg. In other cases, anti-HBc continued to rise for a relatively long period, indicating continued synthesis of HBcAg. This is supported by the detection of HBcAg, a marker for infectivity and for continued synthesis of
Dane particles (Magnius & Espmark, 1972; Takahashi et al. 1976; Imai et al. 1976) in the sera during the same time interval.

The course of hepatitis B infections may be modified by administration of either interferon or of interferon inducers (Greenberg et al. 1976; Desmyter et al. 1976; Purcell et al. 1976). However, in most cases, anti-HB, titres in serum did not decrease after such treatment indicating sufficient synthesis of HBcAg to stimulate a continued immune response. Therefore, the probability that the interferon-treated subjects will eliminate HBcAg may be less than for individuals clearing anti-HBc rapidly, as suggested by our results.

Anti-HBc consists of antibodies with relatively high isoelectric points. This is in agreement with the relatively low isoelectric point of HBcAg (pH 4.4; Howard & Zuckerman, 1977) and the general rule of an inverse relationship between the net electric charge of antigens and their corresponding antibodies (Sela, 1966).

The dose response curves of anti-HBc originating from HBcAg- and anti-HBc-positive sera differed significantly not only for the intact immunoglobulins but also for the corresponding F(ab)2 fragments. Therefore, this dissimilarity cannot be explained by the possible presence in anti-HBc, positive sera of rheumatoid factors (Kacaki, Siem & Brouwer, 1977; Tedder & Briggs, 1977) reacting with the Fc portion of IgG and possibly enhancing the reaction between HBcAg and anti-HBc (Ashe & Notkins, 1966). The distinct dose response curves may rather be attributed to a relatively low affinity of anti-HBc from HBcAg-positive sera for antigenic determinants on HBcAg. As mentioned before, HBcAg positivity is associated with serum infectivity and with continued synthesis of Dane particles and their nucleocapsid component. On the other hand, anti-HBc positivity is accompanied by greatly diminished production of HBcAg. The corollary of our findings is the probable association between the biosynthesis of HBcAg and the avidity of antibodies to HBcAg, suggesting that they may either be involved in the modulation of HBV infections, or that their properties may merely reflect the immune competence of the host. It also seems plausible that a portion of anti-HBc is used up in the formation of antigen–antibody complexes in individuals continuously producing HBcAg. In such circumstances, the proportion in serum of unbound anti-HBc, with low avidity could increase.

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