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The affinity of anti-HBc antibodies in acute and chronic hepatitis B infection

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SUMMARY

Antibodies to hepatitis B core antigen (anti-HBc) are found in the sera of all individuals infected with hepatitis B virus. A role for these antibodies has been suggested in determining the outcome of infection. In this study, the affinity of anti-HBc antibodies in asymptomatic virus carriers was compared with that of antibodies present in the sera of patients with chronic liver disease. Persistently infected individuals with no evidence of clinical disease were found to have anti-HBc antibodies of greater affinity, compared with the chronic liver disease group. Sera from patients with chronic hepatitis contained high levels of low-affinity antibody whereas antibody levels in asymptomatic carriers were significantly lower. These findings are discussed in relation to the predicted role of anti-HBc antibodies in mediating hepatitis B virus-related hepatocellular injury.

Keywords hepatitis B virus antibody affinity hepatitis B core antigens

INTRODUCTION

Immune responses to the hepatitis B virus (HBV) nucleocapsid have been characterized in patients with acute and chronic hepatitis B. Antibody to a major immunodominant determinant site on the intact structure (HBcAg) appears early during the acute illness and may be the only serological marker of recent or ongoing virus replication. Recent data suggest that a cellular response directed against HBcAg may also be important in the pathogenesis of the disease. This is suggested by the finding that autologous cytotoxic lymphocytes are sensitized to determinants expressed by the nucleocapsid gene C (Mondelli *et al.*, 1982, Iwarson *et al.*, 1985). This response is thought to be enhanced late in the acute phase by an elevation in the number of HLA class I molecules expressed on the hepatocyte surface (Pignatelli *et al.*, 1985; Thomas & Scully, 1985). Paradoxically, immune responses to HBcAg may also be significant in immunity to HBV. Immunization of chimpanzees with either recombinant-derived or liver-derived HBcAg particles results in protection against HBV challenge (Murray *et al.*, 1984; Iwarson *et al.*, 1985). The basis of this protection is unknown, although prior sensitization of mice with HBcAg or synthetic peptide analogues can potentiate B cell responses to the outer surface coat (HBsAg) determinants on subsequent exposure to HBV (Milich *et al.*, 1987).

The nucleocapsid of HBV consists of a 19000–22000 mol. wt major polypeptide expressed by the nucleocapsid gene C. There is some disagreement as to the exact site of initiation codon for this protein and the amino-terminal sequence of the naturally occurring molecule is unknown. A change in antigenicity to an *e*-antigen positive state occurs *in vitro* after treatment of the nucleocapsid with ionic detergents (Mackay, Lees & Murray, 1981), and *in vivo* a similar antigenic specificity (HBeAg) is detected serologically in patients supporting high levels of HBV replication. This results from proteolytic cleavage of the carboxyl DNA-binding sequence of a 22000 mol. wt precursor molecule inserted into cellular membranes via an additional hydrophobic signal sequence at the amino terminus (Ou, Laub & Rutter, 1986).

Previous studies have shown that differences exist in both the level and affinity of anti-HBs antibodies in patients with chronic liver disease compared with individuals with acquired immunity (Brown *et al.*, 1984). In the present study, the nature of the antibody response to HBV nucleocapsid has been studied in patients with chronic hepatitis B and asymptomatic HBV carriers.

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MATERIALS AND METHODS

Sera

Sera from nine chronic hepatitis B patients and seven asymptomatic carriers were studied. All were positive for HBsAg and anti-HBc antibodies.

Assays

Total anti-HBc antibodies were quantified initially at a 1:100 dilution using an ELISA (Organon Hepanostika) and the results

were expressed as the optical density (OD) at 495 nm. A rabbit anti-HBc positive serum was used as a positive control and three normal human sera as negative controls.

Affinity determinations

Recombinant HBcAg expressed in *Escherichia coli* (Biogen, Geneva, Switzerland), was used as a source of antigen. One-hundred microgrammes were labelled with 200 μCi of ^{125}I by the chloramine-T method (Hunter & Greenwood, 1962).

Anti-HBc binding of the radiolabelled HBcAg was determined prior to the affinity assay by the addition of appropriate concentrations of labelled core antigen (25 pmol based on a mol. wt of 19000) to serial dilutions of serum samples. After incubation for 2 h at 4°C, the percentage of antibody-bound antigen was determined following binding of antibody and antibody-bound antigen to protein A–sepharose 4B.

The affinities of anti-HBc antibodies were determined using a double isotope radioimmunoassay as described by Brown *et al.* (1984) using protein A–sepharose 4B to precipitate antibodies and antibody-bound antigen; 10 μl of test sera were incubated with a range of ^{125}I-HBcAg (8–90 pmol) in a total volume of 50 μl. Antibody bound and free antigen concentrations were determined after precipitation with protein A–sepharose 4B. Results were calculated using the following modification of the Langmuir equation (Brown *et al.*, 1984):

$$\frac{1}{b}=\frac{1}{K}\times\frac{1}{Ag}+\frac{1}{Abt}\times\frac{1}{Abt}$$

where b represents the concentration of bound antigen; Ag represents the concentration of free antigen; Abt represents moles of antibody binding sites; and K is the affinity constant expressed in units of M^{-1} (Brown *et al.*, 1984).

Detection of anti-HBc antibodies by ELISA

Microtitre plates were coated with recombinant HBcAg expressed in *E. coli* at a concentration of 10 μg/ml in 0·1 M bicarbonate buffer, pH 9·6. After an overnight incubation at room temperature excess sites were blocked with a 0·25% solution of gelatin for 1 h at 37°C. Sera for assay were diluted in phosphate-buffered saline (PBS)–0·05% Tween 20 and incubated in the antigen-coated wells for 2 h at 37°C. After washing in PBS–0·05% Tween 20, anti-human IgG peroxidase conjugate was added for 2 h at 37°C in order to detect surface-bound anti-HBc antibodies. IgG subclasses were detected by use of appropriate animal anti-human IgG subclass-specific conjugates (kindly provided by Dr M. Devey).

RESULTS

Estimated binding of antibodies to HBcAg

Sera selected for this study were first examined for their ability to bind to ^{125}I-HBcAg. The percentages of labelled antigen bound by antibodies in the sera diluted from 1:10 to 1:40 are shown in Table 1. Sera obtained from uninfected persons gave background values in the range of 8 to 12%. In contrast, sera from infected individuals with anti-HBc antibodies bound up to 80% of available labelled antigen, similar to the level of binding observed with the rabbit hyperimmune serum to HBcAg. On the basis of these findings, a dilution of 1:10 or 1:20 was chosen for the affinity determinations.

Table 1. Binding of ^{125}I-HBcAg to anti-HBc antibodies

Sample	Dilution	^{125}I-HBcAg bound (%)
Rabbit	Undiluted	81·2
	1:10	19·8
Patient 3	1:10	70·1
	1:40	26·9
Patient 4	1:10	80·1
	1:40	59·3
Patient 10	1:10	79·2
	1:40	25·6
Control 1	1:10	9·2
Control 2	1:10	11·8
Control 3	Undiluted	9·0

HBcAg, hepatitis B core antigen.
Patients were hepatitis B virus-infected.

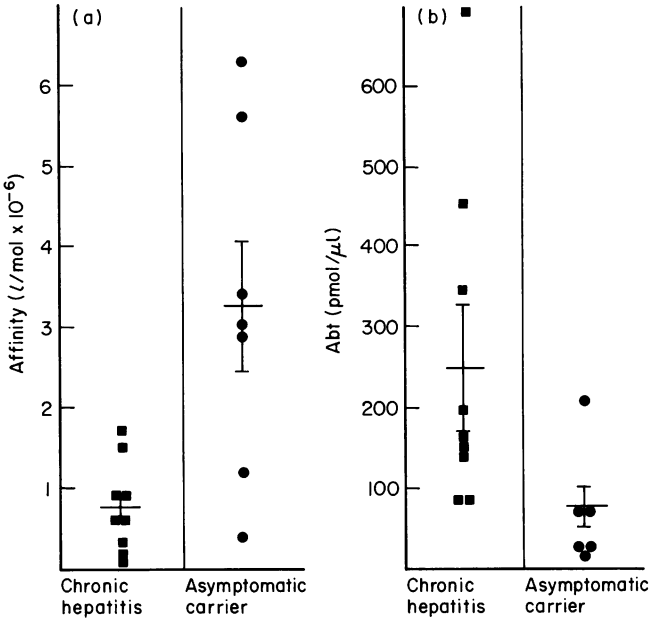

Fig. 1. Affinity (a) and Abt values (b) of anti-HBc antibodies in chronic hepatitis B patients and asymptomatic carriers. The mean values for affinity were $0{\cdot}76\times10^{6}$ M^{-1} and $3{\cdot}26\times10^{6}$ M^{-1}, respectively, and Abt measurements 258 and 72 pmol per μl, respectively. Mean values are shown as bars $\pm$ 1 s.e.m.

Affinity of anti-HBc antibodies for antigen

The affinity values of positive antibody samples ranged from $0{\cdot}1\times10^{6}$ to $6{\cdot}3\times10^{6}$ M^{-1}, representing a 60-fold difference in affinity between the individuals included in this study (Fig. 1). The mean affinity of anti-HBc antibodies in asymptomatic carriers was higher than that in chronic hepatitis B patients. However, individual variations in the affinity of antibodies for HBcAg in the groups was also evident. Furthermore, Abt values in sera from the chronic hepatitis patients (range 83–698) were significantly higher than Abt values in sera from asymptomatic carriers (range 16–210).

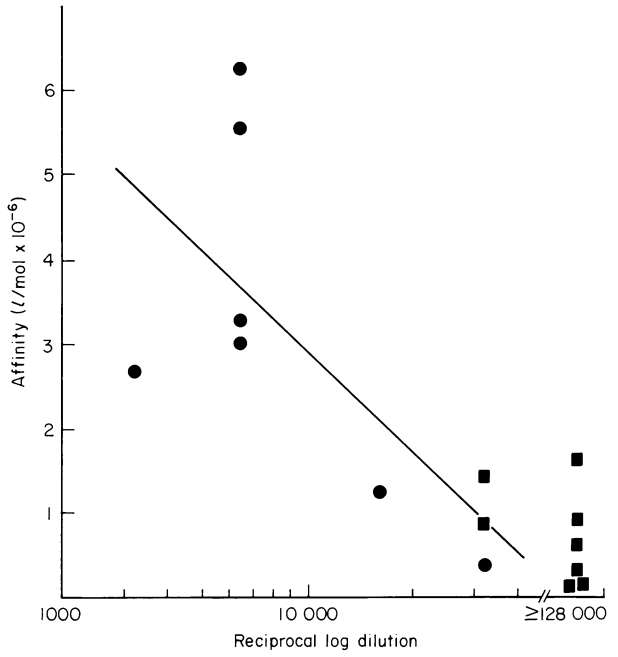

Fig. 2. Correlation between affinity measurements and titre of anti-HBc antibodies as determined by ELISA. ●, asymptomatic carrier; ■, chronic hepatitis.

Correlation between affinity and titre of anti-HBc antibodies

The titres of anti-HBc antibodies were determined by solid-phase immunoassay and compared with the previously determined affinity measurements (Fig. 2). Antibody titres varied between 1:800 and ⩾1:128 000. These results confirm the observation that sera from patients with chronic hepatitis contained high levels of low-affinity antibody.

The IgG subclass profiles were also examined in each group. With one exception, anti-HBc antibodies in all samples was exclusively IgG1. The remaining sample, from a chronic hepatitis patient, also contained IgG4 anti-HBc antibodies (data not shown).

DISCUSSION

The virion of viral hepatitis B consists of an internal 27-nm nucleocapsid with one or more conformationally dependent antigens. Anti-HBc antibodies have been associated with active virus replication. Recently, a possible role of immune responses to HBcAg in protection against hepatitis B has been suggested (Prince, 1984; Stephen, Prince & Brotman, 1984; Iwarson *et al.*, 1985), and the modulating effect of anti-HBc antibodies in virus persistence and maternal transmission has been postulated (Alexander & Eddleston, 1986). However, all studies were based on the measurement of titres and subclasses of anti-HBc antibodies or on T cell-mediated immune responses. It is now widely recognized that to study antibody responses or to assess the roles played by antibodies, it is desirable to determine both the level and affinity of specific antibody as evidence suggests that the affinity of antibody markedly influences its biological properties (Steward & Steensgaard, 1983). Data presented here indicate that anti-HBc antibodies from HBV-infected individuals differ in their affinities for HBcAg. There was a significant difference between patients with chronic liver disease ($0{\cdot}76 \times 10^6$ M^{-1}) and those individuals regarded as being asymptomatic carriers ($3{\cdot}26 \times 10^6$ M^{-1}) ($P > 0{\cdot}01$). Furthermore, sera from chronic hepatitis patients contained high levels of low-affinity antibody as assessed by ELISA and by radioimmunoassay. Antibody levels in asymptomatic patient sera were significantly lower. Calculations of affinity were made assuming a mol. wt of 19 000 for the HBcAg polypeptide; the 22 000 mol. wt protein reported by Feitelson, Marion & Robinson (1982) could be coded for by the sequences reported for HBV subtypes *adw* and *ayw*, but this is deleted in the other sequences when a smaller reading frame utilizing the next initiation codon would give a smaller 19 000 mol. wt product. The amino-terminal sequence of naturally occurring HBcAg proteins are not known.

Recent observations have shown that administration of anti-HBc monoclonal antibodies to chimpanzees causes a significant reduction in T cell cytotoxicity, and prolongs the period of HBV viraemia together with liver cell damage (Pignatelli *et al.*, 1985). The hypothesis of early production of anti-HBc during infection (Lok *et al.*, 1985) and active transport across the placenta may result in altered recognition and destruction of virus-infected cells.

Brown *et al.* (1984) have reported that the mean affinity of anti-HBs was higher in patients who had recovered from acute disease, compared with a group with chronic liver disease. The affinity of anti-HBc antibody in pooled human anti-HBs immunoglobulin has been assumed as $4\text{–}8 \times 10^7$ M^{-1} (Brown *et al.*, unpublished data), ten times higher than that measured in asymptomatic carriers in this study. It remains to be determined whether there is a general tendency to produce antibodies of low affinity in patients with chronic hepatitis, and whether the affinity of anti-HBc could alter in the course of the disease. These points merit a further sequential study in a larger number of patients and carriers, perhaps using synthetic peptides for fine analysis affinity responses to HBcAg epitopes.

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