Viral antigens and antibodies in hepatitis B infection

C. R. HOWARD, A. R. ZANETTI,1 SARA THAL, AND A. J. ZUCKERMAN

From the Department of Medical Microbiology and WHO Collaborating Centre for Reference and Research on Viral Hepatitis, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK

SUMMARY Hepatitis B virus antigens and antibodies were detected in the sera of acute and persistently infected patients. Evidence of active virus replication was confined to immediately before or during the initial detection of hepatitis B surface antigen during acute hepatitis B. Hepatitis B core antibody appeared during the period of antigenaemia and preceded recovery. Hepatitis B e antigen was found in a proportion of sera which contained significant levels of virus particles. In contrast, all sera containing hepatitis B virus particles from persistently infected patients treated by maintenance haemodialysis also contained the e antigen. Among a group of 50 persistent carriers of hepatitis B virus, significant levels of virus production occurred in the presence of antibody to e antigen. In addition, evidence of exposure to hepatitis B virus was found among 3% of blood donors in whose sera the surface antigen was not detected by radioimmunoassay. The significance of these findings is discussed in relation to the aetiology of hepatitis type B.

Sera containing hepatitis B surface antigen (HBsAg) are now known to contain additional antigenic specificities unrelated to the determinants present on the surface antigen. The first of these is the core antigen, so termed because of its close association with the inner component of the 42 nm particle described by Dane et al. (1970). This particle is believed to represent the hepatitis B virus of man. Core antigen may be detected after removal of the outer surface antigen coat either by direct serological examination or indirectly by assay of an integral DNA-dependent DNA polymerase activity (Kaplan et al., 1973). Magnus and Espmark (1972) recognised a third precipitating antigen in some sera containing the surface antigen, which they designated 'e'. The presence of the e antigen appears to correlate with the replication of hepatitis B virus in the host and, in general terms, with varying degrees of liver damage (Nielsen et al., 1974). A significant correlation between the presence of DNA polymerase activity, the presence of hepatitis B e antigen, and infectivity has also been reported, although the significance of the presence of antibody remains unclear. In the present study the presence of hepatitis B core antigen, e antigen, and their respective antibodies were determined in individuals with acute or persistent infection with hepatitis B virus.

Methods

HEPATITIS B SURFACE ANTIGEN AND ANTIBODY DETECTION

Commercially available solid phase radioimmunoassays were used for the detection of HBsAg and its antibody (Austria II, Abbott Laboratories Inc, North Chicago, Ill., USA). Where appropriate, the titre of HBsAg present was expressed as a ratio of the sample counts per minute to the mean value obtained with 10 replicate samples of a confirmed HBsAg-negative serum. All assays were quantified in an LKB Model 1280 gamma-counter to a standard error of less than 4%.

DETECTION OF ANTIBODY TO CORE ANTIGEN

Antibody to hepatitis B core antigen (HBCAg) was carried out essentially as described by Howard and Zuckerman (1977). Trace labelled HBCAg was prepared from a concentrated hepatitis B virus fraction obtained from the serum of a chronically infected chimpanzee. Sera were tested at dilutions of 1/5 and 1/50, and immune complexes were separated either by binding to staphylococcal protein A or by the addition of donkey anti-human IgG (Wellcome Reagents Ltd, Beckenham, Kent, UK). Samples that precipitated more than 50% of the added trace label were scored as positive.

DETECTION OF E ANTIGEN AND ANTIBODY

Hepatitis B e antigen (HBeAg) and its antibody were
detected either using the gel immunodiffusion method as described by Magnius and Espmark (1972) or by the use of gel rheophoresis plates (Abbott Laboratories Inc, North Chicago, Ill, USA). All positive samples were confirmed by their serological identity to reference reagents held at the WHO Collaborating Centre for Reference and Research on Viral Hepatitis.

**ELECTRON MICROSCOPY**

Examination of sera and concentrated pellets of hepatitis B antigens was performed essentially as described by Krugman et al. (1974a).

**DETECTION OF CORE ANTIGEN-ASSOCIATED DNA POLYMERASE ACTIVITY**

DNA polymerase activity in sera was quantified by a modification of the method originally described by Kaplan et al. (1973). Sera were concentrated by centrifugation overnight at 28,000 × g in an AR40-3 rotor or for four hours at 66,000 × g in an SW65 rotor. After resuspension into a 1/20th volume of PBS, Nonidet P40 and 2-mercaptoethanol were added to a final concentration of 0.7% and 0.2% respectively. The reaction mixture contained 16 μmol tris pH 7.5, 4 μmol MgCl₂, 12 μmol NH₄Cl, 5 nmol each of dATP, dCTP, and dGTP, and approximately 35 pmol of ³H-thymidine methyl-5-triphosphate at 45-50 Ci/mmol (Radiochemical Centre, Amersham, Bucks, UK). After a two-hour incubation at 37°C two 50-μl volumes were spotted onto 3MM Whatman filter discs and dried at 60°C for 20 minutes, and each disc was individually treated with two 50-μl volumes of 5% trichloroacetic acid over 18 hours. Discs were then dehydrated in methanol, dried, and immersed in a toluene-based scintillation fluid for counting.

**Results**

**DETECTION OF CORE ANTIGEN, E ANTIGEN, AND ANTIBODIES DURING ACUTE HEPATITIS B INFECTION**

The appearance of core antigen-associated DNA polymerase activity as a marker of replication of the virus was investigated in a total of 109 sera collected serially at seven-day intervals from 43 patients with acute hepatitis B admitted to hospital. The amount of acid-insoluble product as a measure of DNA polymerase activity over a two-hour-period of incubation is shown in Figure 1. Each result is expressed as a ratio of the counts per minute (cpm) over the background control.

![Figure 1 DNA polymerase activity in sera obtained from 43 cases of acute hepatitis B: samples containing e antigen ○ and e antibody □.](http://jcp.bmj.com/ on September 15, 2023 by guest. Protected by copyright.)
obtained with respect to that obtained with similarly treated negative control material. A ratio 2 or greater was regarded as being significant. The vertical bar on the right shows the range of values obtained with samples prepared in a similar manner from patients with acute viral hepatitis in whom HBsAg was not detected by radioimmunoassay. The highest values of DNA polymerase activity were recorded during the first two weeks of observation.

The data obtained from two patients are shown in more detail in Figure 2. In patient (a) e antigen was still detectable in the absence of significant DNA polymerase activity. Its disappearance was closely followed by the detection of core antibody, but no antibody to the e antigen could be detected. In patient (b), although a similar rapid decline in the titre of the surface antigens was observed, e antibody was detected and appeared slightly ahead of the core antibody even though the latter was detected by a sensitive radioimmunoassay procedure. The appearance of e antibody suggests that in this case e antigen must have appeared much earlier in the infection before serum samples were available for examination.

**Hepatitis B Viral Antigens in Patients on Maintenance Haemodialysis**

A correlation was sought between the core antigen associated DNA polymerase and e antigen in the sera of 16 patients undergoing maintenance haemodialysis for chronic renal failure. All had been persistent carriers of HBsAg for at least 12 months. A close correlation was found between significant levels of DNA polymerase activity and the presence of e antigen as detected by immunodiffusion (Fig. 3). The antigen was consistently found in the sera from 12 patients (75%), 11 of which also contained high levels of DNA polymerase activity. In contrast, no significant incorporation was detected either in the presence of e antibody or in those samples in which e antigen or its antibody could not be found. In all cases a close correlation was also found between the level of DNA polymerase activity and the presence of virus particles as seen by electron microscopy.

**Incidence of Hepatitis B Virus Antigen in Healthy Blood Donors**

A total of 50 asymptomatic carriers were examined for the presence of DNA polymerase, e antigen, and antibodies to hepatitis B virus antigens. The e antigen was found together with significant levels of DNA polymerase activity in only one instance (Fig. 4). However, in four carriers significant levels of enzyme activity were found in the presence of e antibody. Examination by electron microscopy of sera containing e antibody revealed intact virus particles in both the presence and absence of significant DNA polymerase levels (Fig. 5). In the remaining group of 16 sera, no evidence of e antigen or its antibody was found although five additional sera contained significant levels of DNA polymerase.

Subsequent donations from several of these carriers suggested that there was some fluctuation in the level of DNA polymerase over periods of several months. During the course of these studies blood was regularly obtained from an asymptomatic chimpanzee carrier of hepatitis B virus for other studies. The results of DNA polymerase estimations on each sample are shown graphically in Figure 6.

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**Fig. 2** Appearance of core and e antibodies during acute hepatitis B in two patients. In case (a) core antibody was detected in the absence of e antibody, whereas in case (b) the appearance of e antibody preceded the detection of core antibody.
Fig. 3  Relationship of DNA polymerase activity and e antigen in 14 haemodialysis patients.

Fig. 4  Relationship of DNA polymerase activity and e antigen in 50 asymptomatic HBsAg-positive carriers.

Fig. 5  Electron microscopy of sera from asymptomatic HBsAg-positive carriers containing e antibody. The enzyme activities were determined as 1832 cpm and 138 cpm for sera (a, left) and (b, right) respectively. × 126 000.
The level of activity varied by as much as a factor of four during a period of 14 months, cyclical variations occurring every four to six months.

The presence of hepatitis B core antibody was also found in all but one of the 50 human asymptomatic carriers (Table). In contrast, only 63% of sera from

| Table Detection of antibody during acute and chronic hepatitis B infection |
|-----------------------------|---|---|---|
|                            | No. tested | anti-HBs positive | anti-HBc positive |
| HBSAg positive             |           |                 |                   |
| Acute hepatitis sera       | 35         | 1 (3%)           | 26 (74%)          |
| Haemodialysis patients     | 11         | 0                | 9                 |
| Asymptomatic chronic carriers | 50      | 5 (10%)          | 49 (98%)          |
| HBSAg negative             |           |                 |                   |
| Late convalescent sera     | 68         | 68 (100%)        | 43 (63%)          |
| Healthy blood donors       | 97         | 1 (1%)           | 3 (3%)            |
| Hyperimmune sera to HBSAg  | 2          | 2                | 0                 |
| Immunoglobulin preparations | 2       | 2                | 0                 |

68 healthy blood donors with surface antibody contained also core antibody. Surprisingly, another 3% of donors without detectable surface antigen and antibody were found to have hepatitis B core antibody. No antibody to core antigen could be found in two preparations of immunoglobulin containing high levels of surface antibody together with two hyperimmune sera prepared in laboratory animals to purified surface antigen which were included as controls.

Discussion

The finding of a DNA polymerase activity within the 42 nm virus particles permitted the development of a unique assay for the presence of the virus. The enzyme is closely associated with an essentially double-stranded circular DNA template (Robinson, 1974), and its presence in serum has been associated with replication of the virus and with infectivity (Alter et al., 1976). Krugman et al. (1974b) examined serial serum samples from volunteers who had been inoculated with the MS-2 strain of hepatitis B virus. Hepatitis B surface antigen was detected four weeks after parenteral inoculation and was followed 10 days later by a rise in serum DNA polymerase activity. Both events preceded elevation of the serum transaminase levels. In a similar study of three patients who received multiple transfusions, Kaplan et al. (1974) found DNA polymerase activity in the serum approximately seven days after the appearance of hepatitis B surface antigen but it disappeared before the peak of transaminase elevation was reached. In the present study, significant levels of DNA polymerase activity were found in 31 of 109 sera obtained from cases of acute hepatitis B. The majority of positive findings were confined to the first two weeks of observation (Fig. 1). In a recent study, Cappel et al. (1977) also found the appearance of DNA polymerase activity to be transient, significant levels of enzyme being detected five days before the onset of clinical hepatitis in persons who became infected in a high-risk setting. No attempt was made, however, to monitor the presence of either e antigen or its antibody.

Among the acute hepatitis sera examined in our study, only three were found to contain both e antigen and DNA polymerase activity. Antibody to e antigen was detected in one instance. The antigen was also detected in three other sera lacking significant DNA polymerase activity, whereas e antibody was present in six sera, although generally confined to the last sample obtained from the patient. In contrast, core antibody was detected as early as the second week in all of 16 patients tested. In several instances, the combined serological findings revealed that e antibody response may precede the appearance of core antibody (Fig. 2). It is noteworthy that e antibody was detected in earlier samples using
relatively insensitive techniques of immunodiffusion whereas core antibody was detected by a radioimmunoassay procedure (Howard and Zuckerman, 1977). In one of the two cases illustrated, e antigen was clearly detected in the absence of DNA polymerase activity.

In contrast, a strong correlation between the appearance of e antigen and circulating DNA polymerase activity was found in 11 persistently infected patients undergoing maintenance haemodialysis. Sera from all these patients contained the 42 nm virus particles when examined by electron microscopy in contrast to two other patients with antibody in the absence of DNA polymerase activity. Nordenfelt and Kjellén (1975) also found a similar correlation between e antigen, DNA polymerase activity, and viral replication in a small number of patients undergoing maintenance haemodialysis. Nielsen et al. (1974) reported that hepatitis B e antigen was significantly more common in patients with chronic hepatitis and cirrhosis and hepatitis B surface antigen than in patients with acute viral hepatitis and suggested that the presence of the e antigen may be a valuable prognostic marker of progression to chronic liver disease. However, the possibility that the appearance of the e antigen is a host response has yet to be definitely excluded (El Sheikh et al., 1975; Perrillo et al., 1977).

Several investigations have been directed to the relationship between the e antigen and replication of hepatitis B virus in asymptomatic persistent carriers since Magnius and Espmark originally suggested that a proportion of blood donors with e antigen may carry a greater risk of transmitting hepatitis B infection. In a study of over 400 such blood donors in Japan, Imai et al. (1976) found that 14% had circulating hepatitis B e antigen and a further 24% possessed e antibody. Findings of specific DNA polymerase activity were confined to sera with e antigen, and a further 15% of the remaining samples were found to be free of both antigen and antibody. In the present study a total of 50 persistent carriers were similarly tested. The sera from 10 of these (20%) contained significant levels of DNA polymerase activity, but e antigen was found concurrently in only one carrier. A further four were found to have e antibody in the presence of the enzyme, and electron microscopy confirmed the presence of hepatitis B virus particles in two of these. Neither e antigen nor its antibody could be detected in the remaining samples with significant DNA polymerase activity. Resolution of this group requires the development of more sensitive techniques for the detection of e antigen and its antibody. Liver biopsies were not available in order to assess the extent, if any, of liver damage in either category. Feinman et al. (1975), however, reported an association of e antibody with a normal or slightly abnormal liver, but they did not relate this to the presence of circulating HBV.

In agreement with previous reports (Tsuda et al., 1975; Hoofnagle et al., 1977), almost all of the persistent carriers investigated possessed hepatitis B core antibody. In the present study a group of 97 healthy volunteer blood donors in whose serum the surface antigen was not detected by radioimmunoassay were also examined. Of these, 3% were found to contain core antibody in the absence of all other indicators of infection with hepatitis B virus. Other studies have also indicated the possibility of recent or ongoing infection, hepatitis B taking place in certain individuals in the absence of detectable surface antigen. Tsuda et al. (1975) reported that 3% of blood donors in Japan had core antibody as detected by an immune adherence haemagglutination method in the absence of surface antigen and antibody. Gerber et al. (1977) found core antibody in 21% of 33 patients with surface antigen-negative chronic hepatitis, and Irwin et al. (1977) have recently reported that 39% of surface antigen-negative acute hepatitis patients in a military population had core antibody. It would therefore seem likely that active replication of this virus may occur in some patients in the absence of excess production of the surface antigen in the form of 20 to 25 nm particles. The lack of excess surface antigen-bearing material may lead to the failure of sensitive radioimmunoassay procedures to detect material that may contain only circulating complete virus particles. It has been suggested that infection with hepatitis B virus may proceed in a manner that results in a large number of defective particles necessary for the establishment and subsequent maintenance of virus persistence (Gerin et al., 1975). This hypothesis is strengthened by the finding of apparently empty 42 nm virus particles and the fluctuations in DNA polymerase activity observed over a period of time in the one persistently infected chimpanzee. This may represent an underlying mechanism of defective particle production that is not dissimilar to the proposed mechanism of vesicular stomatitis virus persistent infection in tissue culture (Sinarachatanant and Huang, 1977).

The study of antigens and antibodies associated with hepatitis B virus in acutely and persistently infected individuals is of immense value in the understanding of pathogenesis of this infection. The association of e antigen with infectivity and the possible significance of the production of core antibody in exposed persons will be essential factors in the evaluation of the experimental hepatitis B vaccines currently under development.
We are indebted to Miss Jill Preece for excellent technical assistance.

The hepatitis research programme is supported by the World Health Organisation, the Medical Research Council, the Wellcome Trust, and the Department of Health and Social Security. One of us (ARZ) was in receipt of a CNR award during the course of this work.

References


Requests for reprints to: Professor A. J. Zuckerman, Department of Medical Microbiology, London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London WC1E 7HT.